

AN IMMUNOGENETIC AND VIRAL STUDY OF
ALZHEIMER'S DISEASE

BY

EDWARD BERNARD RENVOIZE

MB, ChB, (Edinburgh), DPM (Leeds), MRCPsych.

A THESIS

Submitted for the Degree of Doctor of
Medicine in the University of Edinburgh, 1982.



CONTENTS:

PREFACE

Page

| | |
|----------------------|----|
| Introduction | 2 |
| Aims of the study | 5 |
| Summary of the study | 6 |
| Acknowledgements | 11 |

REVIEW OF THE LITERATURE

| | |
|--|----|
| Clinical features of presenile dementia of the Alzheimer type | 14 |
| Clinical features of senile dementia of the Alzheimer type | 17 |
| Pathological features of presenile and senile dementia of the Alzheimer type | 21 |
| Aetiology of Alzheimer's disease | 24 |
| The relationship of Alzheimer's disease to normal ageing | 28 |
| The genetics of presenile dementia of the Alzheimer type | 32 |
| The genetics of senile dementia of the Alzheimer type | 36 |
| The genetics of Alzheimer's disease : conclusions | 39 |
| Age-related changes in the immune system | 41 |

| | Page |
|--|------|
| The immune system and the brain | 46 |
| Immune studies in Alzheimer's disease: | |
| 1) Brain antibody studies | 49 |
| 2) Humoral immunity | 50 |
| 3) Complement system | 51 |
| 4) Cell-mediated immunity | 51 |
| 5) Amyloidosis | 52 |
| Slow virus infections of the central nervous system: | |
| Introduction | 58 |
| Subacute sclerosing panencephalitis | 63 |
| Progressive multifocal leukoencephalopathy | 71 |
| Kuru | 75 |
| Creutzfeldt-Jakob disease | 81 |
| Viruses and Alzheimer's disease | 87 |
| The cytomegaloviruses | 92 |
| The HLA system | 98 |
| ABO and rhesus blood groups and disease | 107 |

METHODS

| | |
|---------------------------|-----|
| Patients: | |
| a) Case finding | 114 |
| b) Selection criteria | 114 |
| c) Assessment of patients | 117 |

| | Page |
|--|------|
| d) Identification of secondary cases of Alzheimer's disease | 118 |
| e) Follow up of patients | 119 |
| Serology: | |
| a) Specimens | 119 |
| b) HLA typing | 119 |
| c) ABO and Rh blood grouping | 120 |
| d) CMV antibodies | 120 |
| Post-mortem studies | 120 |
| Virus isolation attempts | 121 |
| Controls: | |
| a) HLA antigens | 121 |
| b) ABO and Rh blood groups | 122 |
| c) CMV antibody study | 122 |
| Statistical procedures | 123 |
| The roles of the workers engaged in the study | 125 |

RESULTS

| | |
|---|-----|
| 1) General information on the patients studied | 127 |
| 2) General characteristics of the patients and controls for the CMV study | 127 |

| | Page |
|---|------|
| 3) ABO blood group frequencies in the patients and controls | 128 |
| 4) Rhesus blood group frequencies in the patients and controls | 128 |
| 5) HLA antigen frequencies in the patients and controls | 129 |
| 6) HLA-B15 and clinical characteristics of the patients | 130 |
| 7) Family studies | 131 |
| 8) CMV antibody titres in the patients and controls | 132 |
| 9) Post-mortem studies | 133 |
| 10) Virus isolation attempts | 133 |
| 11) Creutzfeldt-Jakob disease in an HLA-B15 positive 15 year old girl | 172 |
| 12) Presenile dementia of the Alzheimer type in one of monozygotic twin sisters | 174 |

DISCUSSION

Methodology:

| | |
|---|-----|
| 1) The clinical differentiation of Alzheimer's disease from other forms of dementia | 193 |
| 2) The patient population studied | 198 |
| 3) Identification of secondary cases of Alzheimer's disease | 199 |
| 4) The choice of the control groups | 200 |

| | Page |
|--|------|
| 5) Blood grouping and HLA tissue-typing | 202 |
| 6) Analysis of results | 203 |
| Results: | |
| 1) ABO blood group results | 205 |
| 2) Rhesus blood group results | 207 |
| 3) HLA antigen results | 212 |
| Theoretical mechanisms to explain the association of HLA-B15 and Alzheimer's disease | 216 |
| 4) HLA-B15 and clinical characteristics | 230 |
| 5) Family studies | 232 |
| 6) The relationship of Alzheimer's disease to the myeloproliferative disorders and Down's syndrome | 235 |
| 7) CMV antibody results | 238 |
| Possible mechanisms by which Alzheimer's disease could result from persistent CMV infection | 242 |
| 8) Creutzfeldt-Jakob disease in an HLA-B15 positive 15 year old girl | 249 |
| 9) Presenile dementia of the Alzheimer type in one of monozygotic twin sisters | 252 |
| <u>CONCLUSIONS</u> | 255 |
| <u>APPENDICES</u> | 259 |

REFERENCES

PREFACE

INTRODUCTION

Senile dementia and Alzheimer's disease are the commonest forms of dementia occurring in the senium and presenium respectively. They have devastating effects on sufferers and their families, and as the proportion of the elderly population increases, serious economic consequences for the health and social services of the country.

The relationship of Alzheimer's disease to senile dementia has been debated since Alzheimer first described the disease in 1907 (1-3). Alzheimer thought that the disease he described represented a premature form of senile dementia but Kraepelin considered that it was a disease *sui generis*. However most clinical, neuropathological and genetic studies support the theory that they constitute a single disease entity rather than representing two distinct diseases.

Recent studies (1,3,4) have found that the clinical features are very similar in both disorders. Traditionally the presence of parietal lobe features such as apraxia, agnosia and aphasia, have been considered to be characteristic of Alzheimer's disease, and rare in senile dementia, but recent studies cast doubt on this teaching. Lauter and Meyer (3) found a high incidence of parietal lobe features in senile demented, and McDonald (5) also confirmed their presence in senile dementia, although he found that they tended to occur in

the younger patients, and perhaps conferred a poorer prognosis.

Sourander and Sjogren (6) argue that Alzheimer's disease and senile dementia are separate disorders because they are distinguishable in terms of intensity of neuropathological changes. However the pathological features of both disorders are essentially identical (1,3,4) making their histological differentiation impossible in many cases without a clinical history.

Support for a genetic distinction between Alzheimer's disease and senile dementia rests on the findings of Larsson, Sjogren and Jacobsen (105), who reported no cases of Alzheimer's disease in over 2000 first degree relatives of senile demented. However a careful reading of their results reveals that 10 secondary cases of dementia occurred in the presenile age group. The results of other genetic studies (shortly to be reviewed), and the report by Stam and Op den Velde (7,8) of an association of the haptoglobin Hp¹ gene with Alzheimer's disease and senile dementia, do not support a genetic distinction.

Therefore it is difficult not to accept Newton's conclusion (1) that there are no clear grounds for distinguishing between Alzheimer's disease and senile dementia except by the arbitrary limits of age, and perhaps rate of progression and severity of neuropathological changes, and that both diseases are examples of the same disorder. As a recognition of this unitary

concept, the terms presenile dementia of the Alzheimer type (PDAT) and senile dementia of the Alzheimer type (SDAT) have been proposed (9), and will be used in the remainder of this thesis. When the term Alzheimer's disease (AD) is used, it will refer to PDAT and SDAT, and not specifically to the form of the disease occurring before the age of 65.

For various reasons there has been little basic research into AD, and its aetiology is unknown. However the increased risk of developing AD in the close relatives of a minority of patients suffering from this disorder, implies that genetic factors may be of aetiological importance in some cases. AD also has features which suggest that immunological factors may be involved in its pathogenesis, although few studies have attempted to identify their nature.

It has recently been recognised that a number of slowly progressive diseases of the central nervous system, previously thought to be heredo-degenerative disorders, are caused by slow virus infections. As yet, there have been few studies which have examined the possible involvement of viruses in the aetiology of AD although this disease has some histological and biochemical features which are compatible with a viral aetiology.

A number of human diseases, some of which are thought to be immunologically mediated, are associated with particular HLA antigens, ABO and Rhesus blood

groups, and such studies have helped to clarify some of the genetic and immunological factors operating in these disorders.

Human cytomegalovirus (CMV) is a common virus which may rarely cause neurological disease associated with primary infection. Following primary infection the virus becomes latent in tissues or cells which are as yet unidentified. Reactivation of CMV is a well recognised phenomenon and it is conceivable that such reactivation may cause neurological disease. The possible involvement of the CMV in AD is suggested by a Swedish study (366) which found that demented patients had higher antibody titres to CMV than functionally-ill psychiatric patients.

The principal aims of the study were:

- 1) To identify possible genetic and immunological factors in AD, by studying ABO and Rhesus blood groups, and HLA antigens, in patients suffering from this disorder.
- 2) To clarify the nosological relationship between PDAT and SDAT using these genetic markers, and by family studies.
- 3) To determine if any HLA antigen, found to be significantly associated with AD, influences the

clinical features of the disorder.

- 4) To investigate the possible role of CMV in the aetiology of AD.
-

Summary:

Survey of Literature:

This covers:

- 1) Some psychiatric, pathological and aetiological aspects of PDAT and SDAT, with particular reference to genetic, immunological and viral studies.
 - 2) The relationship of AD to normal ageing, and the age-related changes in the immune system.
 - 3) Slow virus infections of the central nervous system, with particular reference to subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, kuru and Creutzfeldt-Jakob disease.
 - 4) The CMV, the HLA and the ABO and Rhesus blood groups.
-

The Study:

A study of 37 inpatients suffering from PDAT and 87 inpatients suffering from SDAT is described. The case histories of a female patient suffering from PDAT, with an identical twin discordant for the disease, and a 15 year old girl with Creutzfeldt-Jakob disease, are also described.

The frequencies of the ABO and Rhesus blood groups, and the principle HLA-A and -B antigens were determined in the patients, and compared with the respective frequencies in a group of hospital staff and blood donors drawn from similar geographical catchment areas as the patients. Serum antibody titres to CMV were also determined in the patients and a similarly aged control group of 39 non-demented psychiatric inpatients for comparison.

The next of kin of 34 of the study patients were interviewed, and the cases of AD occurring in first degree relatives identified.

On completion of the study, the data was drawn together and analyzed.

The Main Findings of the Study:

- 1) The ABO blood group frequencies of the

patients did not differ significantly from two control groups of Leeds blood donors.

2) SDAT patients showed a slight but significant increase in the frequency of the Rhesus negative blood group compared with controls (relative risk 1.8, $P < 0.05$).

When this result is combined with data from similar reported studies using Woolf's method of statistical analysis (521,522), the association of SDAT with the Rhesus negative blood group is highly significant ($P = 0.0002$).

3) Patients suffering from PDAT and SDAT showed a significant increase of HLA-B15 (PDAT : relative risk 3.0, $P < 0.005$; SDAT : relative risk 2.3, $P < 0.005$).

When these results are combined with data from similar reported HLA studies of AD using Woolf's formula, the association of AD with HLA-B15 remains statistically significant ($P = 0.0015$).

4) The frequency of HLA-A2 was non-significantly increased in the PDAT and SDAT patients. However the combination of this result with data from other studies using Woolf's method, shows a statistically significant association of AD with HLA-A2 ($P = 0.04$).

- 5) As in other similar studies, this study found a small non-significant increase in the frequency of HLA-B40 in patients with AD.
- 6) There were no significant differences in the frequency of HLA-B15 in male and female patients.
- 7) No significant differences were found in mean age at onset, or duration of disease, when HLA-B15 positive and negative patients were compared.
- 8) No significant difference was observed in the frequency of AD among first degree relatives of HLA-B15 positive and HLA-B15 negative patients matched for sex and age at onset of disease.
- 9) Most of the secondary cases of AD were dead, and only two could be personally examined. The two secondary cases of AD were relatives of HLA-B15 positive patients, and both were also HLA-B15 positive. Cases of PDAT and SDAT were found to occur within the same families.
- 10) Patients and controls did not differ significantly in the prevalence of serum antibody titres, or serum geometric mean titres to CMV.
- 11) HLA-B15 positive patients showed a

significantly higher serum geometric mean titre to CMV compared with HLA-B15 negative patients.

12) A histological diagnosis was obtained in 18 patients. AD was confirmed in 15 patients, and mixed AD - multi-infarct dementia in 2 further cases. Multi-infarct dementia was diagnosed in another patient, and he was excluded from the study.

13) Biopsy specimens of the brain, liver and kidney from 8 patients were inoculated into human embryonic lung fibroblasts but failed to induce the cytopathic effects characteristic of CMV.

The results of the study are discussed in relation to the findings of other studies, and various mechanisms are proposed by which the above immunological and viral factors may be of aetiological importance in the pathogenesis of AD.

Acknowledgements

I am grateful to the Consultant Psychiatrists at High Royds Hospital, Menston, Scalebor Park Hospital, Burley-in-Wharfedale, Stanley Royd Hospital, Wakefield, and Lynfield Mount and Northern View Hospitals, Bradford, for allowing me to study their patients. I am grateful to the nurses and staff of the medical records departments at the above hospitals for their cooperation, and also to the relatives of patients who participated in the study.

I should also like to thank the following: Dr. S.M. Rajah and his staff at the Leeds Regional Blood Transfusion Centre for the HLA typing and blood grouping; Dr. M.H. Hambling and his staff at the Leeds Public Health Laboratory for the virology studies; Dr. D. Harriman and his colleagues at the Department of Neuropathology, Leeds General Infirmary, for the post-mortem studies; Dr. G. Bevans at St. James' Hospital, Leeds, for statistical advice; Leeds University Department of Photography for the photographs; and Mrs. G. Boland for typing the thesis.

The study was carried out when the writer was a Senior Registrar and later Research Fellow in the Department of Psychiatry, University of Leeds. I am indebted to Professor R.H.S. Mindham for his encouragement and support throughout the study, and acknowledge the financial support of the British Foundation for Age Research during part of the study.

It is a particular pleasure to acknowledge the advice and helpful criticisms of Dr. D.M. Weir, Reader, Immunology Laboratory, Department of Bacteriology, University of Edinburgh Medical School, who was my adviser during the study, Professor J.C. Woodrow, Department of Medicine, Liverpool University Medical School, and Dr. P.O. Behan, Reader in Neurology, Institute of Neurological Sciences, Glasgow.

REVIEW OF THE LITERATURE

CLINICAL FEATURES OF
PRESENILE DEMENTIA OF THE
ALZHEIMER TYPE

CLINICAL FEATURES OF PRESENILE DEMENTIA OF THE ALZHEIMER
TYPE (PDAT):

PDAT is characterized by a slowly progressive mental deterioration with impairment of memory, disorientation, and confusion, leading eventually to profound dementia, starting before the age of 65 (6,10-12).

It is by far the most common of the presenile dementias, and females are apparently more often affected than males (13). Sjogren et al (14) reported a combined morbidity risk of 0.1% for PDAT and Pick's disease, and Terry (13) estimated a prevalence rate of 0.3% for PDAT.

The onset is insidious and usually starts between the ages of 50 and 60 years. Three main stages are typically described.

In the first stage, lasting 2-3 years, memory begins to fail, and the patient becomes disorientated in time and place. The ability to cope with everyday activities is lost, and mood disturbances such as perplexity, agitation or apathy may occur.

There is a progressive deterioration of intellect and personality, and aphasia, apraxia, agnosia and acalculia may be seen reflecting parietal lobe involvement by the disease process. Extrapyrarnidal disorders can occur in the form of disturbance of posture, increase in muscle tone, and other parkinsonian features as a result of lesions in the basal ganglia and frontal lobes, and the patient may become deluded and hallucinated.

Finally the patient sinks into the bedridden terminal stage of profound dementia, with total memory loss, emaciation, double incontinence, limb contractures, and frequently seizures. Death usually occurs from bronchopneumonia within 5-10 years of the onset.

CLINICAL FEATURES OF SENILE
DEMENTIA OF THE ALZHEIMER
TYPE

CLINICAL FEATURES OF SENILE DEMENTIA OF THE ALZHEIMER
TYPE (SDAT):

Roth (15) defines SDAT as:

"a condition with a history of gradual and continually progressive failure in the common activities of everyday life and a clinical picture dominated by failure of memory and intellect and disorganization of a personality, where these were not attributable to specific causes such as infection, neoplasm, chronic intoxication or cerebrovascular disease known to have produced cerebral infarction."

SDAT develops insidiously after the age of 64, and occurs more commonly in women (16). Kay et al (17) reported a prevalence rate of 4.2% for SDAT, and 3.9% for multi-infarct dementia in the Newcastle region. They also observed that in half the cases the severity of mental deterioration was similar to that usually found in demented hospital patients, and that over 80% of the patients suffering from dementia were still living at home. The results of other prevalence studies agree broadly with the Newcastle findings (16-20). The prevalence of SDAT rises steeply with age, and is about eight times as common in the over 75's as those aged between 65-75 years (21).

The typical signs and symptoms of the disease (15, 22, 23) are essentially identical to those of PDAT:

The first sign is usually impairment of memory for

recent events. Further memory deterioration is accompanied by disorientation and previous personality traits may become exaggerated, sleep disturbance is common, and acute confusional states may be seen superimposed on the underlying dementia.

Once established, factors such as intercurrent illness, drugs, medical procedures such as lumbar puncture or pneumoencephalography, a sudden change in environment, or the loss of a spouse, can adversely affect the progress of the disease.

Agitation, depression and paranoid ideation are common as intellect and personality continue to decline. As the disease advances, there is typically disinhibition, blunting of emotion, deterioration of personal habits, loss of sphincter control, and the appearance of repetitive futile behaviour such as rocking movements.

However McDonald (5) found evidence of clinical heterogeneity, and was able to classify patients into two groups on the basis of parietal lobe involvement. Patients with preserved parietal lobe function were significantly older and had a much better six-month prognosis than those with deteriorated parietal lobe function.

In the terminal stages of the disease, the patient rapidly loses weight despite adequate food intake, and usually dies within a few years from the onset due to an intercurrent infection. At post-mortem the viscera and body are often atrophied, possibly because of the involve-

ment of the hypothalamus, and its connections to the limbic system, by the disease process (24).

PATHOLOGICAL FEATURES OF PRESENILE
AND SENILE DEMENTIA OF THE
ALZHEIMER TYPE

PATHOLOGICAL FEATURES OF PRESENILE AND SENILE DEMENTIA OF
THE ALZHEIMER TYPE:

Corsellis (25) states that PDAT cannot be distinguished from SDAT on histological grounds, "all the differences being of emphasis or of quantity rather than intrinsic". Therefore the description of the neuropathological features which follows applies both to PDAT and SDAT, although they are said to be more severely developed in the former.

There is usually generalized cortical atrophy, often with emphasis on the frontal and temporal lobes, and the ventricles are frequently enlarged (25,26). Degeneration of neurons and loss of dendritic spines (27), particularly in the outer three cortical layers are extensive, and senile plaques, neurofibrillary tangles and granulovacuolar degeneration are present throughout the cortex, with emphasis on the hippocampal and amygdaloid regions of the limbic area (28-36). The marked degeneration in the limbic system is of particular interest, as this area of the brain has been shown to play an important role in emotion, behaviour, memory and intellect.

The senile plaques (28,30-36) are roughly spherical areas of disintegrating tissue ranging from 5 μ to 100 μ in diameter, which are found scattered throughout the cerebral cortex, and their origin is a matter of considerable debate. Several types of plaque

which differ in structure have been described (219). The typical senile plaque consists of a central amyloid core, surrounded by a ring of filamentous material containing degenerative neuronal processes, post-synaptic boutons, macrophages, microglia, reactive oligodendroglia, degenerating mitochondria, and astrocytes.

The neurofibrillary tangles (29,30,32-36) found in the cell bodies of neurons and their processes, are composed of bundles of paired 10 nm helical filaments (36-38).

Evidence suggests that the senile plaque amyloid and the paired helical filaments contain protein with a similar β -pleated sheet structure.

Granulovacuolar degeneration (35) - the formation of membrane-bound vacuoles each containing a central clump of finely granular material - are found mainly in the hippocampal pyramidal cells. Affected neurons may also show an accumulation of lipofuscin pigment (35).

Amyloid deposits (congoophilic angiopathy) (39) are also found in the cerebral arteries, and arterioles, the adventitia of veins and venules, and the basement membranes of capillaries.

The subcortical grey matter is much less severely affected, and senile plaques are not found in the white matter. However the latter may show axonal degeneration, astrocytic hyperplasia, and fibrous gliosis.

AETIOLOGY OF ALZHEIMER'S DISEASE

AETIOLOGY OF ALZHEIMER'S DISEASE:

The aetiology of AD is unclear. Human and animal studies suggest that the cholinergic system of the brain is important in memory function (40-42). The activity of choline acetyltransferase, a key enzyme in the synthesis of the neurotransmitter acetylcholine, is reduced in AD brains (43-46). It has therefore been suggested that the disease is associated with the failure of cholinergic transmission, but attempts to increase the concentration of brain acetylcholine by the administration of precursors such as choline (47-52), lecithin (53-55) and physostigmine (55,56) have led to equivocal results. Recently a far wider disturbance of neurotransmitter systems has been postulated, with the dopamine, gamma-aminobutyric acid, and noradrenergic systems all affected (57).

Neurofibrillary tangles of the paired helical type have been found in the brains of patients suffering from a variety of diseases (58,59), which suggests either that they can be induced by a wide variety of pathological agents, or are a non-specific reaction in chronically-diseased brains. Neurofibrillary tangles have been reported in viral diseases, in infections such as tuberculosis, scarlet fever and dysentery, in the punch drunk syndrome, and in other disorders in which chromosomal abnormalities, inborn errors of metabolism, and metal intoxications are implicated (59). Curiously

patients with Down's syndrome who survive into middle age are predisposed to the development of a presenile dementia which is pathologically similar to AD (60-62).

The results of cytogenetic chromosomal studies of peripheral lymphocytes in patients with AD have been conflicting (63-70), and it is unclear if chromosomal abnormalities during life play any aetiological role in the disease. However the observation by Galloway and Buckton (71) of a loss of chromosome 21 from cultured peripheral blood cells in an ageing population, could be important if viruses were implicated in AD. Whalley and Buckton (62) have speculated that as chromosome 21 may be necessary for human interferon to be effective, the loss of this chromosome in sufficient numbers of small lymphocytes in old people could result in defective interferon production, and increased susceptibility to infection.

Aluminium can also induce neurofibrillary tangles in experimental animals (73,74), although they differ morphologically from the tangles found in AD. Crapper et al (75) found raised brain aluminium levels in AD and suggested that this metal might have a neurotoxic role in the disease. This finding is of interest as some patients undergoing chronic renal dialysis develop an encephalopathy which has been attributed to aluminium intoxication (76,77), although neurofibrillary tangles are absent from the brain. However McDermott et al (78,79) found no differences in aluminium brain concentrations between mentally normal patients and patients with AD, and

suggested that high aluminium levels are related to age rather than dementia. Further doubt on the aetiological role of aluminium has been cast by Delaney (80) who reported that spinal fluid levels of aluminium are low in AD patients.

There is clear evidence that genetic factors are implicated in both PDAT and SDAT. The age-related changes in the immune system, and the immunological abnormalities found in AD by some workers also suggests that immune factors may be involved in the aetiology of AD. The recognition of human slow virus infections of the central nervous system, has also prompted speculation that viruses might play an aetiological role in AD. The evidence in support of a genetic, immunological and viral aetiology will shortly be examined.

THE RELATIONSHIP OF ALZHEIMER'S
DISEASE TO NORMAL AGEING

THE RELATIONSHIP OF ALZHEIMER'S DISEASE TO NORMAL AGEING:

The relationship of AD to normal ageing is unclear.

Kral (81,82) has attempted to define two types of senescent memory impairment. In the benign form, subjects are able to recall the main features of a past experience, although have difficulty in recalling specific names and places. Both sexes are equally affected, and the subjects retain insight into their forgetfulness, which deteriorates only slowly. However in Kral's malignant form subjects are unable to recall the main features of a past experience. It occurs more commonly in women, progresses rapidly leading to behavioural disturbances, and death rates are higher and survival times shorter than in the benign group. This form of memory impairment is associated with SDAT.

However the demonstration of two types of senescent memory impairment does not necessarily imply that they result from two separate pathological processes. It is also possible that both are the outcome of a single process, which if mild results in the benign form, or if severe the malignant type.

Neuropathological evidence does suggest a link between AD and normal ageing. The senile plaques, neurofibrillary tangles and granulovacuolar degeneration of AD, are also found in the brains of the majority of healthy people over 65 years of age who show no evidence of dementia (83-85,87). These changes are relatively

uncommon in people under 65, but thereafter increase in frequency with age (85).

The senile plaques and neurofibrillary tangles appear to have an identical structure in AD and normal ageing. However they are found in much greater abundance in AD, and the degree of dementia is quantitatively correlated with their numbers (83,84,86,87). The Newcastle group (83,84,87) found a threshold effect for senile plaques, beyond which some degree of dementia usually occurs, and Roth (84) concludes that on present evidence, SDAT is the result of an acceleration of the changes associated with senescence.

Recent biochemical studies have yielded conflicting results about the relationship of AD to normal ageing. Bowen et al (88,89) reported a marked reduction of choline acetyltransferase (CAT) activity in AD but not in histologically normal brain from non-demented elderly people. However Perry and her colleagues (90), while agreeing that CAT activity is significantly reduced in AD, also found a decrease in normal individuals with advancing age. They reported that CAT activity decreased significantly as the mean senile plaque count rose in both AD and normal old people (91-93).

The increased risk of developing AD in first degree relatives of affected patients is frequently used to support the distinction between AD and normal ageing. However both conditions could result from an identical pathological process initiated by environmental factors. The genetic predisposition to AD could operate by

increasing individual susceptibility to these factors or by determining a more severe pathological reaction to them.

THE GENETICS OF PRESENILE
DEMENTIA OF THE ALZHEIMER TYPE

THE GENETICS OF PRESENILE DEMENTIA OF THE ALZHEIMER TYPE:

The evidence for the genetic transmission of PDAT has been reviewed by Pratt (94,95), Slater and Cowie (96), and Jarvik (97).

Since 1929 there have been many reports of families in which 2 or more members suffered from PDAT (69,98).

Feldman et al (98) reported a family with 13 affected relatives in 4 generations, but failed to establish linkage of the hypothetical PDAT gene with abnormal chromosomes, blood group antigens and finger print patterns. The onset of symptoms usually developed between the ages of 30 and 40, and the disease was histologically confirmed in 4 cases.

Wheelan (99) described a family in which 6 relatives in 2 generations had PDAT. Blood grouping was carried out on 31 members of the family to test for possible linkage between the hypothetical disease gene and blood group genes, but results were inconclusive.

Heston and his colleagues (100) reported a family in which 19 people were affected in 4 generations. Histological examination of tissue from 4 brains confirmed the disease. The age at onset of symptoms ranged from 22 to 52 years with a mean of 37 years, and early onset may be more common in the familial than the sporadic forms of PDAT.

Landy and Bain (101) described a family in which 3 out of 6 siblings and their mother developed PDAT, the disease

being confirmed by cerebral biopsy in 2 of the siblings. Interestingly their father and maternal grandfather also probably suffered from SDAT.

In some cases of familial PDAT features such as muscle twitching, spastic paraplegia and amyloidosis of the cerebral vessels are reported, and unlike the sporadic form there is no female preponderance (95).

The sporadic forms of PDAT, in which a positive family history is exceptional, are said to form the large majority of cases, and other workers have studied the frequency of dementia in the families of such patients.

In a Swedish study, Sjogren, Sjogren and Lindgren (14) studied the relatives of 36 PDAT patients, in 18 of whom the diagnosis had been histologically verified. They found that 3 parents and 2 siblings suffered from a similar presenile dementia, and furthermore 2 parents and 1 sibling had SDAT. Unfortunately no histological results were available to confirm the clinical diagnosis in the secondary cases. In this study, allowing for age at death, the morbidity risk of developing dementia was 10% for parents and 3.8% for siblings.

In a Swiss study (4) of 97 PDAT patients, which did not involve field work, the proportions affected with PDAT and SDAT respectively were for parents 1.4% and 2.8%, for siblings 3.3% and 0.47%, and for children 1.6% and 0.8%.

Cases of SDAT among the relatives of patients with PDAT were also found by Lauter and Meyer (3).

Heston and Mastri (102,103) investigated the

relatives of 30 patients with a clinical and histological diagnosis of PDAT, and found 22 secondary cases among 301 first degree, and 556 second degree relatives. A post-mortem confirmed the diagnosis in 6 of the secondary cases. In 2 of the confirmed secondary cases, the patients were aged 70 and 76 at the earliest possible onset of the disease. They also reported a significantly increased incidence of Down's syndrome (trisomy 21) and myeloproliferative disorders (leukaemia, Hodgkin's disease, lymphosarcoma, multiple myeloma) among the relatives. These results are particularly interesting in view of the high proportion of patients with Down's syndrome who develop Alzheimer's disease if they survive into their late thirties (60-62), and also their greatly increased chances of developing leukaemia (104).

Heston and Mastri (102,103) speculate that micro-tubular organisation could be adversely affected by a genetic defect, causing a predisposition to Alzheimer's disease, trisomy 21, and myeloproliferative diseases.

THE GENETICS OF SENILE
DEMENTIA OF THE ALZHEIMER TYPE

THE GENETICS OF SENILE DEMENTIA OF THE ALZHEIMER TYPE:

This subject has been well reviewed by Pratt (94,95), Slater and Cowie (96), and Jarvik (97).

It has long been recognized that genetic factors are implicated in SDAT. In 1925, Meggendorfer found 18 secondary cases of SDAT among first degree relatives of 60 patients with histologically confirmed SDAT (96). Weinberger in 1926 found a higher incidence of SDAT among the relatives of senile demented than in the general population, and later Cresseri made similar findings (96).

The largest and most systematic study of the genetics of SDAT was made by Larsson, Sjogren and Jacobson (105) in Sweden. They investigated the genealogy of the first degree relatives of 377 SDAT patients which involved field work in the case of 256 families.

They found a total of 60 secondary cases: 17 among the parents (unaffected 703), 37 among the siblings (unaffected 1577), 5 among the spouses (unaffected 300), and 1 among the children (unaffected 786). On the basis of these findings, Larsson, Sjogren and Jacobson estimated that the morbidity risk for SDAT was increased 4.3 times among siblings and parents of patients with the disease, compared to the general population at corresponding ages, and there was steady approximately linear increase of the morbidity risk with age. The increased incidence among relatives was not due to their greater expectation of life, as they had no more than average longevity.

The Swedish workers found no enhanced morbidity risk for other psychoses, and claimed that no cases of PDAT or Pick's disease occurred among the relatives. This claim is curious as in 10 of their secondary cases, dementia started before the age of 65.

Because of the apparent absence of intermediate forms between SDAT and normal senescence among the unaffected relatives, Larsson, Sjogren and Jacobson proposed that SDAT was determined by a single major autosomal gene with partial penetrance carried by 12% of the population.

A group of Swiss workers (4) also found that the frequency of SDAT in the first degree relatives was about four times that found in relatives of patients with multi-infarct dementia. Of 229 SDAT patients, the proportions affected with SDAT and PDAT respectively were for parents 2.22% and 0%, for siblings 3.4% and 0.43%, and for children 3.24% and 2.16%.

Further evidence that genetic factors are clearly important in the disease has been presented by Kalman et al (106), who studied 108 pairs of twins in which the index twin suffered from SDAT. The incidence of the disease was 42.8% in the monozygotic twins, compared with 8% in the dizygotic twins, 6.5% in the siblings, and 3.4% in the parents.

THE GENETICS OF ALZHEIMER'S
DISEASE : CONCLUSIONS

Conclusions:

- 1) The increased risk of developing the disease in the close relatives of some patients with PDAT and SDAT, and the higher concordance rate for SDAT in monozygotic than in dizygotic twins, is strong evidence that genetic factors predispose to AD.
 - 2) The exact mode of inheritance is unclear, and there may be genetic heterogeneity. The results of single family pedigree studies suggest that the familial form of PDAT is transmitted as a regularly manifested autosomal dominant trait. However the results of the studies into the sporadic forms of PDAT and SDAT are less easily interpreted, and are consistent with two genetic modes of transmission : autosomal dominant inheritance with low penetrance, or multifactorial inheritance.
 - 3) The occasional occurrence of both PDAT and SDAT in first degree relatives within individual families supports the hypothesis that the presenile and senile forms of AD share a common genetic predisposition.
 - 4) However the lack of full concordance for SDAT in monozygotic twins indicates that non-genetic factors are of aetiological importance in AD.
-

AGE-RELATED CHANGES IN THE
IMMUNE SYSTEM

AGE-RELATED CHANGES IN THE IMMUNE SYSTEM:

The increased prevalence of the disease with age, and the presence of identical neuropathological features in many non-demented elderly subjects, has led to speculation that AD results from an acceleration of the changes associated with normal ageing (107). One theory of ageing links the process to changes in the immune system, as there is considerable evidence that immune functions decline with age.

The primary lymphoid system (spleen, thymus, and lymph nodes) undergoes atrophy after puberty which continues throughout adult life into old age (107-111). Paradoxically however, there is an accumulation and accentuation of lymphoid tissues in the bone marrow, salivary and thyroid glands, lung parenchyma and liver with increasing age (107,108), and lymphoid cell infiltrations have also been found in the ageing brains of hamsters (112) and humans (113).

Mackay (108) postulates that the increase of this lymphoid tissue is the result of pathological events such as autoimmune reactions in response to changes in cellular proteins with age.

Humoral immunity, as measured by circulating antibody titres to a range of bacterial and viral antigens, and the primary response to extrinsic antigens, gradually declines with age (107-109,111,114-116). This decline is thought to be partly due to age-related intrinsic deficiencies in the cells directly involved in antibody

formation (116). However serum immunoglobulin (Ig) G and IgA levels are increased (117-119), and there is also an increased incidence of paraproteinaemia with age (119-122). The proportion of bone marrow derived lymphocytes (B cells) is increased in the peripheral blood (109, 123-127), but it is not clear if there is a corresponding increase in absolute numbers.

However the major cause for the impairment of immune function with age is the decline in cell-mediated immunity (CMI). Pre-existing and primary delayed hypersensitivity-type reactivity declines (107-109,125,128-130), and hyporesponsiveness may be associated with reduced life expectancy in old age (130). The percentage of peripheral thymus dependent lymphocytes (T cells) is decreased (123-127, 131,132), which appears to be due to a drop in actual numbers. T cell function probably declines (108,109,117, 124,125,130,133,134), and the proportion of null cells (lymphocytes that lack T and B cell receptors) are increased (135).

The decline in CMI may be mediated by the involution of the thymus gland (107-109,136), which appears to play an important role in ageing. Neonatal thymectomy in mice results in impairment of CMI, autoantibody formation, wasting and death, but has minimal effect on Ig levels except Ig A which decreases (109). The capacity of the murine thymus to influence T cell maturation also declines with thymic involution (137).

As humoral and cell-mediated immunity decline with age,

there is an increase in the prevalence of autoantibodies (107,108,117,124,138-142). Nandy (143-145) reported a progressive increase in the accumulation of brain-reactive antibodies in the sera of mice and humans as a function of age. A serum gamma globulin fraction which binds to cytoplasmic constituents of neurons in sections of human brain tissue, with increased frequency and intensity with age, has also been described (146). Field and Shenton (147) reported that ageing in mice and humans is associated with the emergence of new antigenic determinants in the tissues similar to those found in scrapie mouse brain and spleen, although this finding has not been confirmed.

The increased frequency of autoantibodies and the diminished antibody responses with age, may be related to the decline in suppressor T cell activity (148-150).

Amyloidosis, which is thought to have an immune aetiology, is frequently found in old age (151-155), particularly in the brain (153), heart (153,154,156-161), seminal vesicles (153,158) and pancreas (153,154,162).

Schwartz and his colleagues reported that senile plaques are commonly associated with cardiac and pancreatic amyloid in the senium, and proposed that this 'senile amyloidotic triad' has a common origin from an underlying metabolic disorder (153,163,164).

Franklin et al (165) found a progressive age-related increase of serum amyloid A protein in normal people which rose steeply after the age of 70, although Ignaczak

et al (166) were unable to confirm this. This led Benson and his colleagues (167) to propose that 'senile' amyloid may be of the secondary type, but evidence suggests that senile amyloid differs according to organ localization (168-170).

These age-related immunological abnormalities have led Burnet (171) and Walford (107,172) to propose their immune theories of ageing which relate the process to the accumulation of cell mutations, and the failure of the immune surveillance system to eliminate them.

THE IMMUNE SYSTEM AND
THE BRAIN

THE IMMUNE SYSTEM AND THE BRAIN:

Under normal circumstances, the blood-brain barrier which separates blood from the brain and cerebrospinal fluid (CSF), limits the entry of proteins and their diffusion into the intercellular space (173,174,175).

The central nervous system (CNS) lacks an organized lymphatic system, and lymphocytes are scarce as indicated by the low cell count in normal CSF (173,176). However the relatively privileged immunological state of the brain breaks down in some degenerative and infectious diseases of the nervous system, when antibody, complement, macrophages, lymphocytes and other cells can enter from the blood stream to initiate immune responses (173,176).

Raised immunoglobulin levels in the CSF may be secondary to increased serum antibody levels, as in the paraproteinaemias, and also to enhanced permeability following damage to the blood-brain barrier as a result of disease (173-174).

However raised CSF immunoglobulin levels may reflect their synthesis by lymphoid cells within the CNS, as in disseminated sclerosis, and SSPE (173,174,176). These antibodies show as discrete oligoclonal bands in the globulin region on electrophoresis (176).

IMMUNE STUDIES IN
ALZHEIMER'S DISEASE

IMMUNE STUDIES IN ALZHEIMER'S DISEASE:1) Brain antibody studies:

A brain specific antibody was detected in patients with SDAT by Czech workers, (cited in reference 113), but Whittingham et al (177) failed to confirm these findings.

Mayer, Chughtai and Cape (178) reported that SDAT females showed an excess of antineuronal antibodies compared with SDAT males, or controls.

More recently Nandy (145) found a higher titre of brain reactive antibodies in PDAT and SDAT patients than in age- and sex-matched controls.

Tkach and Hokama (179) found higher serum titres of brain antibody in 3 patients with 'chronic brain syndrome' compared with controls, but Ingram, Phegan and Blumenthal (146) found no difference in the frequency of neuronal antibody between a similarly described group of patients and controls. Unfortunately the term 'chronic brain syndrome' was inadequately defined in both studies, and therefore the relevance of these results to AD is unclear.

Differing clinical criteria, and variations in techniques for determining antibodies, may be partly responsible for these conflicting results.

Brain reactive antibodies (BRA), if confirmed, could play a primary or secondary role in AD. A breach of the blood-brain barrier in AD could allow the entry of pre-existing circulating BRA or provoke its formation, leading to neuronal damage as the result of antigen-

antibody reaction. Nandy (144-145) has described a similar mechanism of brain damage in the mouse following damage to the blood-brain barrier.

However BRA could also be secondary to the pathological process of AD, and have no aetiological significance in the disease.

2) Humoral immunity:

Behan and Feldman (180) reported lower serum albumin and a diffuse increase in serum immunoglobulins in PDAT and SDAT patients, similar to that found in patients with primary and secondary amyloidosis. Kalter and Kelly (181) reported similar findings.

Recently Cohen et al (182,183) detected increased serum Ig G and Ig A levels in patients with SDAT and multi-infarct dementia, and a PDAT group, compared with controls. Eisdorfer and Cohen (184) found that SDAT patients with lower total serum immunoglobulin concentrations had significantly lower cognitive test scores, and concluded that the relative hyperimmunoglobulinaemia along with the progressive drop of immunoglobulin levels with greater impairment of cognition, suggested that aberrant immune functions may be characteristic of these disorders.

Tavolato and Argentiero (185) reported normal serum levels of Ig A and Ig G, but decreased Ig M and reduced

numbers of B lymphocytes in 11 patients with PDAT.

Mayer, Chughtai and Cape (178) reported normal serum immunoglobulin levels in SDAT patients.

Studies of serum autoantibodies in PDAT patients have failed to find any abnormalities (181,185).

However the recent detection of oligoclonal bands in the CSF of patients with PDAT (186,187) supports the hypothesis that abnormal immunological processes may be involved in the disease although these may be secondary rather than primary.

3) Complement system:

No differences in serum complement levels between AD patients and controls were reported by Mayer et al (178) and Tavolato and Argentiero (185). Changes in the frequencies of the C3 groups of the complement system in PDAT, were found by Mehne et al (188).

4) Cell-mediated immunity (CMI):

Tavolato and Argentiero (185) reported an insignificant decrease in the numbers of T lymphocytes, and a significant increase in the numbers of null cells in PDAT, compared with controls.

Eisdorfer, Cohen and Buckley (182) found a delayed



cutaneous hypersensitivity to skin antigens in 9 out of 13 patients with PDAT compared with controls, but skin tests to determine T-cell functions were normal in a further study (185).

The results of 3 in vitro studies to determine T cell function using lymphocytes from the peripheral blood of AD patients, have been similarly conflicting (185,181, 187).

5) Amyloidosis:

The term amyloidosis covers a group of disorders with different clinical features, but which result from amyloid deposition into organs and tissues (107,165,189-198).

Amyloid formation is the end product of many diseases, and is thought to be the consequence of impairment of the immune system. The immune theory is supported by the results of animal experiments and the association of amyloidosis with diseases which have immune abnormalities.

The presence of cerebral amyloid in AD further suggests that immune factors may be implicated in the disease.

Amyloidosis has been traditionally classified into primary and secondary forms. Primary amyloidosis occurs without antecedent or coexisting disease, and is frequently associated with monoclonal immunoglobulins and Bence-Jones proteins (165,195,199). Amyloid associated with

neoplasms involving plasma cells or lymphocytes also closely resembles the primary form (165,195).

Secondary amyloidosis is associated with a variety of chronic infections, and connective and neoplastic diseases in which there is persistent antigenic stimulation with excessive immunoglobulin production (165,195). Non-specific changes involving the gamma or alpha globulin fractions are sometimes found in the serum (165).

The brain is rarely involved in primary or secondary amyloidosis (200), and nervous system involvement is usually confined to the peripheral and autonomic nervous systems (201-204). However Haberland (200) described a case of a 49 year old woman with primary systemic amyloidosis with cerebral involvement who had clinical features compatible with a diagnosis of AD.

Primary and secondary amyloid consists of two main components : helical fibrils made up of protein polymers which constitute 90% of the amyloid substance, and the plasma (P) component, a glycoprotein which migrates as an alpha - globulin by electrophoresis (165,194,197,205).

The amyloid fibrils have a similar electron microscopic appearance, but differ in their main protein components. In primary amyloidosis, the fibrils are composed of whole or fragments of immunoglobulin light polypeptide chains, probably derived from a circulating (immunoglobulin light chain) precursor (194,206-208). In the secondary form, the fibrils are composed of the amyloid A (AA) protein (194,197,209,210) unrelated to

immunoglobulin, and which is thought to be derived from a serum precursor called serum AA (SAA) (195-197,211).

Elevated SAA levels have been found in patients with a variety of acute and chronic infections, connective and neoplastic diseases, in secondary amyloidosis, and also in the primary type of amyloidosis associated with multiple myeloma (165,166,195).

Scheinberg and Cathcart (213) have proposed a unified concept of amyloid disease in which both primary and secondary amyloidosis share common pathogenetic pathways, with macrophage activation representing the initial step.

The primary and secondary amyloid fibrils, although differing in their main protein components, share an identical twisted β - pleated sheet configuration which gives them their property of congo-red staining (197,214). Amyloid A protein and immunoglobulin light chain fragments have also been found together in primary and secondary amyloidosis (215).

The final mechanism of amyloid formation may involve digestion of the precursor protein by proteolytic enzymes (194,197,216), although other mechanisms are possible.

Glenner (198,216,217) identifies 3 types of cerebral amyloid deposits in AD : congophilic angiopathy, senile plaques and paired helical filaments.

Congophilic angiopathy has been reported in a variety of neurological disorders and in non-demented old people but is particularly associated with AD (39,218).

An association between amyloid-rich senile plaques and the severity of congophilic angiopathy was reported by Mandybur (39). Glenner (217) has recently proposed that AD is caused by intra-cortical congophilic angiopathy which by compromising the blood-brain barrier allows a chronic influx of neurotoxic serum proteins into the brain. He thinks that the most likely source of the amyloid in congophilic angiopathy is from the serum, but there could also be a local protein synthetic abnormality in the cerebral vasculature leading to the local production of an amyloidogenic compound.

Amyloid is an important component of senile plaques, although it is sometimes absent. Horst et al (219) have suggested that there are 4 consecutive phases in their formation, with the plaques containing no amyloid representing the terminal phase.

The origin of plaque amyloid is unknown. Divry and more recently Schwartz (153,164) and Horst et al (219) have proposed that brain amyloid is a manifestation of a generalized senile amyloidosis, and that amyloid is the direct toxic cause of neuritic degeneration and senile plaque formation. However amyloid is not neurotoxic and Wisniewski (36) postulates that plaque amyloid is a secondary phenomenon to neuritic degeneration.

Attempts have been made to determine the biochemical nature of senile plaque amyloid. Powers and Spicer (221) found that it resembled apudamyloid, but Bartoli, Antonutto and Bianchi (222) found that it had many

features of immuno - amyloid. Recently Ishii and colleagues (223-224) reported that plaque amyloid in AD contains fragments of immunoglobulins, as found in primary amyloidosis, which strongly suggests that immunological mechanisms are involved in their aetiology.

Glenner (216) has discussed the possible origins of the amyloid core. Amyloid could be synthesized and precipitated locally in the brain as the result of an antigen-antibody reaction following prolonged sensitisation to an antigenic stimulus, for example a virus, as the brain has the capacity for independent immunoglobulin synthesis. Amyloid could also be a synthetic product of the phagocytic cells, or arise from the proteinaceous debris of the degenerated neurites present at the plaque periphery.

Alternatively amyloid could arise following deposition of plasma proteins from the blood stream, as there is evidence of a circulating precursor of immunoglobulin-type amyloid protein. Recently Miyakawa and colleagues (225,226) suggested that senile plaque amyloid is produced by the basement membrane of blood vessels, and speculated that antigen-antibody reactions between the blood and vessels may be important.

The intracellular paired helical filaments also contain protein with the typical twisted β - pleated sheet fibrils of amyloid (198,217,36).

SLOW VIRUS INFECTIONS OF
THE CENTRAL NERVOUS SYSTEM

SLOW VIRUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM:

The concept that some slowly progressive degenerative human diseases of the central nervous system (CNS) may be caused by slow virus infections has been accepted only recently, after kuru and Creutzfeldt - Jakob disease (CJD) were shown to be transmissible diseases (227). However it has long been known that scrapie, a degenerative disease of the CNS in adult sheep, is caused by a virus (228-230).

Gajdusek (231) has described the general criteria of slow viral infections:

1. A long initial period of latency, lasting for several months to several years.
2. An ultimately fatal illness after the appearance of clinical signs.
3. Primary anatomical lesions limited to a single organ system.
4. Noninflammatory pathology.
5. Absence of immune response.
6. Limited range of susceptible hosts.
7. Often a heredofamilial pattern of infection.

Slow virus infections of the CNS caused by unconventional viruses:

Four degenerative diseases of the CNS, grouped under

the generic term 'subacute spongiform encephalopathies' (229,231-239) are now known to be caused by unconventional viruses: scrapie, and transmissible mink encephalopathy in animals, and kuru and CJD in man. They have similar clinical and neuropathological features, and are caused by very unusual viruses with close biological and physical properties (231-235). These diseases have long incubation periods of months or years, and pursue an unremitting progressive course leading to death. Pathology is restricted to the CNS, particularly the grey matter of the brain. The brains show diffuse neuronal loss, neuronal vacuolation, astrocytic hypertrophy and proliferation, leading to spongiform changes (status spongiosus) of the grey matter. In man, amyloid plaques are also sometimes seen.

These diseases are caused by stable viruses which are far more resistant to inactivation by ultraviolet light, and to treatment with formalin or heat, than are conventional viruses. They have never been seen with the electron microscope although they are large enough to be detected. They elicit no inflammatory response in the CNS nor in other organs in which the virus may be present. Patients remain afebrile, and the viruses do not elicit an antibody response in naturally or experimentally infected animals. Humoral and cell-mediated immune responses of the host are normal. The viruses, which are closely associated with membranes and inactivated when such

association is broken, have been propagated in vitro in cell cultures derived from the brains of infected animals, but they do not show any cytopathic effect. The diseases are transmissible to experimental hosts with long incubation periods.

There has been considerable speculation on the nature of these transmissible agents (231,233-235). Suggestions include that they are self-replicating membrane fragments which lack nucleic acid, viroids, satellite viruses which activate or are themselves activated by a helper virus latent in the susceptible host, or are common viruses which have become modified in some way as is thought to happen with the measles virus in patients with subacute sclerosing panencephalitis. It is conceivable that scrapie, transmissible mink encephalopathy, CJD and kuru are caused by a single agent modified by different hosts (232).

Slow virus infections of the CNS caused by conventional viruses:

It has long been appreciated that neurological damage can occur as the result of acute infection with conventional viruses, particularly the arboviruses and herpes simplex virus, but it is now recognized that some

conventional viruses can also produce slow virus infections of the CNS which fulfil many of Gajdusek's criteria (227, 232, 235-238).

Subacute sclerosing panencephalitis (SSPE) and progressive multifocal leukoencephalopathy (PML) are examples of human slow virus diseases caused by conventional viruses which result in progressive degenerative disease of the CNS. However they differ in some respects from slow virus diseases with unconventional viruses (240): the viruses have an RNA or DNA type of nucleic acid, and induce specific immune responses. A virus-associated inflammatory response is evoked in the brain, and the virus can be seen under the electron microscope.

Kuru, CJD, SSPE and PML are rare disorders, but they are important paradigms for the study of other chronic human degenerative CNS diseases of unknown aetiology such as AD.

At first sight, the possibility that a slow virus is implicated in the aetiology of AD seems very speculative. However the disease has some features compatible with such a hypothesis, and it is important to point out that kuru and CJD were also called heredo-degenerative disorders before being recognized as slow virus infections.

These four examples of human slow virus CNS infections will now be reviewed to illustrate the concepts of slow virus disease, and to briefly examine the possible mechanisms by which apparently harmless and ubiquitous

conventional viruses become pathogenic in SSPE and PML.

SUBACUTE SCLEROSING PANENCEPHALITIS

SUBACUTE SCLEROSING PANENCEPHALITIS:

Subacute sclerosing panencephalitis (SSPE) is a rare, progressive, fatal disease of the CNS, which is thought to be caused by measles or a measles-like virus (236-238, 241-246).

The disease occurs predominantly in children and adolescents. In SSPE patients in the United States, Jabbour et al (247) found that the mean age of onset was 7.2 years, with an age range of 2 to 21 years. Canal and Torck (248) found similar results. However, SSPE has also been reported in adults (249).

Freeman (245) has described the typical clinical features and course of the disease. The initial stage is characterized by insidious intellectual and personality deterioration, and later by myoclonic jerks of the extremities, head and trunk. This is followed by further intellectual deterioration and progressive neurological impairment. The myoclonic jerks become more persistent and repetitive, and dysphagia, rigidity, spasticity, cortical blindness and sometimes extrapyramidal movements may occur. In the final stages, there is profound dementia, stupor, and marked rigidity progressing to a decerebrate stage. Disturbances in the autonomic nervous system such as hyperthermia may occur, as a result of hypothalamic dysfunction. Death is usually from a terminal bronchopneumonia.

However the clinical features and course of SSPE can be very variable. About 80% of SSPE patients follow the

relentless progressive course described above, and die within 1 to 3 years of the onset of the disease (246). In a small number of patients, death occurs within a few weeks of the onset of the symptoms (250), but in others the disease can take a much more protracted course over many years in which temporary remissions and short periods of improvement may occasionally be seen (245, 251-253).

The electroencephalogram (EEG) pattern is variable but typically consists of paroxysmal bursts of high-voltage diphasic slow wave complexes recurring at 3.5 to 20 s intervals synchronous in all leads (245,246,254). Frequently the myoclonic jerks are synchronous with these paroxysmal bursts. As the disease progresses the complexes may be followed by flattening of background activity, the so-called 'suppression burst' pattern, but in some patients sporadic sharp waves are seen (245, 246,254).

The pathological features of SSPE have been summarised by Sourander and Haltia (255), Freeman (245) and Agnarsdottir (246). The brain may appear grossly normal but inflammatory changes are widespread throughout the grey and white matter on microscopic examination. They include perivascular cuffing (infiltration of lymphocytes and plasma cells around arteries and veins), leptomeningeal lymphocytic infiltration, microglial cell proliferation, astrocytic hypertrophy, gliosis, neuronal loss, neuronophagia which may lead to cerebral atrophy,

and sometimes demyelination. Eosinophilic intranuclear and intracytoplasmic inclusion bodies are often found in neurons, astrocytes and oligodendroglial cells.

SSPE is an extremely rare disease occurring with a frequency of about 1 per million childhood population, and males are affected about three times more commonly than females (247,248,256). Several reports (248,256) have suggested an association between rural residence and the disease but Jabbour and colleagues (247) could not confirm this. Although two epidemiological studies (247,248) have suggested some geographical variation in the distribution of SSPE cases, there is no solid evidence of temporal or spatial clustering.

No clearcut genetic factors have been identified. Kurent, Terasaki and Sever (257) reported a possible association between HLA W29 and SSPE, but this finding has not been confirmed and family clustering is rarely found (246). Three reports of the occurrence of SSPE in only one of identical twins also argues against a genetic basis (258).

There is considerable evidence that the measles or a measles-like virus is directly implicated in the aetiology of SSPE. In his original descriptions of the disease, Dawson (243,244) suspected a viral aetiology because of the presence of intranuclear inclusion bodies within the degenerating nerve and glial cells of the brains of two patients. In 1965 Bouteille and associates (259), using the electron microscope, found virus-like particles, in

the brains of SSPE patients which resembled the paramyxoviruses. These findings were soon confirmed (260-263). In 1967 Connolly et al (264,265) found high levels of measles virus antibody in the sera and cerebrospinal fluid (CSF) of SSPE patients, and also measles virus antigen in their brains. This was confirmed by other workers (266,267) including Jabbour and Sever (268), and Brody, Detels and Sever (269), who found markedly elevated serum measles antibody titres in SSPE patients, but not in their parents or siblings. These results strongly suggested that the measles virus was involved in the pathogenesis of SSPE, and direct evidence was obtained in 1969 when a measles virus was isolated from cultured brain cells of patients (270,271).

Agnarsdottir (246) and V. ter Meulen et al (272) have reviewed present knowledge of the immune system in relation to the measles virus, and SSPE. Patients appear to have normal cell-mediated and humoral immune responses to antigens unrelated to measles, at least in the initial stages of the disease, but have no in-vivo cell-mediated immune response to measles virus as measured by skin testing (273). SSPE lymphocytes retain their in-vitro capacity to respond to both measles and SSPE-virus-infected cells, although an inhibitory factor present in the sera and CSF of some SSPE patients, has been demonstrated which can block this response (274,275).

Dayan and Stokes (276) found immune complexes of measles antigen, Ig G, and complement, in the brain and

renal glomeruli of a patient who died of SSPE and suggested a possible aetiological role for them in the disease. Measles antigens were also detected in the spleen, liver and lymph nodes from many parts of the body, suggesting that the measles or measles-like virus is disseminated widely throughout the body. The successful isolation of a measles-like virus from lymph node biopsies of some patients with SSPE (277) provided further evidence that infection is not restricted to the brain.

High levels of measles antibodies of the Ig G class are found in the sera and CSF of patients, and some also have detectable measles specific Ig M. (267,278,279). The persistence of measles-specific Ig M suggests that measles virus antigen persists in the SSPE brain, and the increased amount of measles Ig G and Ig M in the CSF as compared to the serum, is evidence that they are synthesized locally within the CNS (278). The abnormally high levels of Ig G found in the CSF, have been shown to consist of oligoclonal bands of antibody directed against different antigenic components of the measles virus (280, 281).

If a preceding measles infection is aetiologically important in the development of SSPE, it is difficult to understand why the disease is so rare, when measles infection is so common. Clearly other factors related to host immunity and viral characteristics are probably involved. For example the majority of SSPE patients develop measles infection unusually early in life, about

50% doing so before 2 years of age compared with about 20% for unaffected children (247,256). The average interval between measles infection and onset of SSPE is approximately 5 years (247). The very young person may perhaps be more susceptible to SSPE following measles infection because of an immature immune system, or an increased susceptibility of the brain cells to virus-induced damage. Brody and Detels (256) speculate that in SSPE patients, measles infection occurs in the presence of passive maternal antibody, which prevents a normal immune response against the measles virus, and allows it to persist.

The SSPE agent probably induces an abnormal immune response in the host which results in disease, but it is unclear whether the agent is in fact the classical measles virus, or a measles-like virus (246,272). There are several possible ways in which the measles virus might cause SSPE. Disease could be initiated by the prolonged presence of classical measles virus in the brain, or develop when measles infection is coupled with another viral infection as proposed by Brody and Detels (256). Alternatively, following infection, the measles virus could become modified in the host during replication. This could result in a mutant neurotropic measles strain which remains latent in the brain cells, being unaffected by high measles antibody levels. Later, reactivation of the mutant virus could occur to produce a slow infection, and subsequently SSPE. A further possibility is that SSPE results from direct infection with an unusual, highly

neurotropic measles or measles-like virus, but the lack of temporal or spatial clustering of SSPE cases makes this unlikely.

PROGRESSIVE MULTIFOCAL
LEUKOENCEPHALOPATHY

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY:

Progressive multifocal leukoencephalopathy (PML) is a rare, subacute, usually progressive demyelinating disease of the brain (236-238,282-284). It usually occurs in association with diseases such as leukaemia, lymphomas, sarcoid, tuberculosis, Hodgkin's disease, and carcinomas which impair the immune system, or following the use of immunosuppressive drugs in the treatment of disorders such as systemic lupus erythematosus or after renal transplantation (284-287). However PML can also occur in the absence of any associated disease (288-290).

PML was first described by Astrom, Mancall and Richardson in 1958 (285), and its main clinical and pathological features have been summarised by Richardson (284). Multiple foci of demyelination are found throughout the white matter of the brain, particularly the cerebral hemispheres, with relative sparing of the axis cylinders. These are surrounded by enlarged oligodendrocytes, many of which contain intranuclear inclusions and abnormal nuclei, and by giant bizarre astrocytes, which often contain mitotic figures and intranuclear inclusions. Less frequently, demyelinating lesions may be found in the grey matter of the cerebral cortex.

Most cases occur between the ages of 50 and 70, the average age being 56 (284). Because of the widespread nature of the lesions, a wide variety of neurological

features may be seen. Typically, they include intellectual and personality deterioration, other mental changes, disturbances of vision, language and gait, and hemiparesis. The EEG usually shows non-specific abnormalities (283): focal slow wave (delta or theta) activity occurs initially which becomes diffuse as the disease progresses. The terminal stage of the disease is characterised by dementia, motor deficits, and finally coma. Death usually occurs within 3 to 6 months of the onset (284), although a few cases have been reported in which the disease has had a more gradual evolution or has undergone remission (291,292).

In 1965 Zu Rhein and Chou (293), and independently Silverman and Rubinstein (294), identified virus-like particles in the oligodendrocytes of PML brains with the electron microscope, which resembled papova virions. Padgett et al (295) in 1971, cultivated a new papova virus from the brain of a case of PML complicating Hodgkin's disease, which they called JC virus after the initials of the patient from whom it was isolated. The JC virus has been isolated from further cases of PML since, and is the papovavirus most frequently associated with the disease (296,297). In 1972 Weiner and associates (298,299) isolated a different papovavirus from the brains of two patients with PML. As this new papovavirus resembled Simian Virus 40 (SV40), it was called SV40-PML.

Gardner et al (300) have also isolated a new

papovavirus from the urine of a renal allograft recipient free from neurological disease and called it BK after the patient. The BK virus has not yet been related to any disease (301). PML is the only human disease in which the JC and SV40-PML viruses have been directly implicated (301). However the JC and BK viruses are oncogenic in some experimental animals (302), and the papovaviruses may also have similar potential in the human brain (303).

Serological surveys indicate that subclinical infections with JC and BK viruses are very common, and mostly occur in childhood (304-306). However antibody to SV40 virus is uncommon in man, and titres are low if present, except in those who have received contaminated poliovirus and adenovirus vaccines, or have had contact with rhesus monkeys (307).

The evidence strongly suggests that PML is caused by an opportunistic papovavirus infection which usually occurs in an immunosuppressed host, and results in the destruction of oligodendrocytes and the demyelination of the brain. A recent study (308) has shown that as well as having a general impairment of cell-mediated immunity, PML patients also show a specific defect in cell-mediated immune response to the JC virus. Whether the disease results from activation of a latent papovavirus in the brain or other tissues, or from viral invasion of the CNS during primary infection, or both, remains undetermined.

KURU

KURU:

Kuru is a progressive fatal disease of the central nervous system (CNS), found only among the Fore people and their neighbours in the Eastern Highlands of Papua, New Guinea (231,232,236-238,309). This region was isolated from the outside world before 1947, and the disease was only first accurately described by Gajdusek and Zigas (310) in 1957.

The word kuru means "shivering" or "trembling" in the Fore language, and accurately describes one of the clinical features of the disease. It occurs predominantly in women and children, and is much less common in adult males. The disease is characterized by a cerebellar ataxia (gait, truncal and limb ataxia), a shiver-like tremor and dysarthria. Other features include pyramidal and extrapyramidal signs, and behavioural changes. As the disease advances a confused and agitated state may develop and in the final stages the patient exhibits gross incoordination, muscle weakness, slurring of speech, dysphagia, urinary and faecal incontinence, and dementia. Death usually occurs within a year of the onset of symptoms. At post-mortem the body is usually wasted, and pneumonia is often the immediate cause of death.

The histopathological lesions are restricted to the CNS, and primarily the grey matter of the brain (311). There is diffuse neuronal loss, with astroglial and microglial hypertrophy and hyperplasia. Vacuoles form in

neurons, and the cerebral cortex takes on a spongy appearance (status spongiosus). Amyloid plaques which resemble those seen in Alzheimer's disease, old age, and scrapie, are found in significant numbers particularly in the cerebellum in two thirds of cases (312). Myelin degeneration mainly involving the spinocerebellar and pyramidal tracts, is another feature, and the walls of the blood vessels may show thickening and perivascular accumulations of mononuclear cells. No pathology is evoked in other organs of the body.

Kuru was at first considered to be a genetically determined disease. There was nothing to suggest that it was caused by a slow virus. The lack of febrile response, the failure to detect any antibody response during the illness, and the absence of acute inflammatory lesions in the brain was against a viral aetiology. However in 1959 Hadlow (313) pointed out the close similarities of the disease with scrapie disease in sheep, which was known to be caused by a transmissible agent with a long incubation period. This prompted further attempts to establish infection as the cause of kuru.

In 1966 Gajdusek, Gibbs Jr., and Alpers (314) demonstrated that chimpanzees inoculated intracerebrally with bacteria-free suspensions of human brain from kuru patients, developed a kuru-like disease 18-30 months later. The clinical and neuropathological features of the disease closely resembled that of human kuru victims, although the areas of brain most adversely affected

varied in the two species. In human kuru the greatest damage was found in the cerebellum and its connections, whereas in experimental kuru the cerebral cortex was most severely affected. Since 1966, primary and serial transmissions of the disease have been successfully achieved in rhesus monkeys, mink and ferrets, as well as chimpanzees (231,315-317). The kuru virus has been found in low titres in the spleen, liver and lymph nodes, as well as in high titres in the brains, of human and chimpanzee kuru victims (231,316,318). The disease has been produced in chimpanzees by the intracerebral, intravenous, intramuscular, subcutaneous and intraperitoneal routes, but not orally, after incubation periods of 11 months to 8.5 years (318).

When first described kuru was responsible for approximately 150 deaths a year, which at that time represented 1% of the total population (310). However in certain Fore tribes the prevalence of active kuru reached 5-10% of the population (310). At one stage 90% of all deaths in adult women were caused by kuru (231).

Gajdusek (232) has suggested that kuru is transmitted by autoinoculation with infected brain at cannibal ceremonies. Apparently ritual cannibalism, the practice of eating one's dead relatives as a rite of mourning, was introduced into the area about 1920, and it has been suggested that kuru may have originated from a rare spontaneous case of Creutzfeldt-Jakob disease that spread through the Fore people because of this custom (232).

Recently a case of Creutzfeldt-Jakob disease was diagnosed and confirmed by cerebral biopsy in a native who lived in the Central Highlands of Papua, New Guinea, about 100 miles from the kuru region (319).

Women conducted the butchery of the dead bodies, and brain tissue was squeezed to a pulp in bamboo cylinders, and then steamed. Apparently adult men rarely participated in this ceremony and seldom ate the flesh of dead kuru victims. Infection probably occurred as the result of conjunctival, nasal and skin contamination with highly infectious brain tissue (231). Since the practice of ritual cannibalism was abandoned, the kuru rates have steadily declined particularly in women and children, and were estimated to have killed about 20 people in 1976. Gajdusek (231) has estimated that kuru will be eliminated by 1988.

Kuru has been observed among people born in the endemic area many years after they migrated away, and the incubation period of the disease appears to vary from 4 to 30 years (318). Attempts to link the kuru agent to known viruses, including CMV, have been unsuccessful (320).

Despite its obvious viral cause, genetic factors have also been thought to be important in the aetiology of kuru (321). Kuru victims commonly have a family history of the disease (310), and it is well recognized that breeds of sheep vary in their susceptibility to scrapie (322). Kitchen and his colleagues (322) reported an association between the Gc Aborigine gene and kuru, but Simmons et al

(323) found no association between the disease and any blood groups of the ABO, MNSs and Rh systems.

CREUTZFELDT-JAKOB DISEASE

CREUTZFELDT - JAKOB DISEASE:

Creutzfeldt - Jakob disease (CJD) was first described by Creutzfeldt in 1920, and independently by Jakob in 1921. The subject has been well reviewed by Kirschbaum (324) and May (325), and Brown and coworkers (326) have recently published an analysis of the clinical features of 124 cases of histologically proven CJD. CJD (236-238,309) is a rare but worldwide progressive and fatal disease of the central nervous system which affects both sexes equally. Most cases occur between the ages of 35 and 65 years with an average age at onset of 60 being reported by Brown et al (326). The prodromal symptoms can be vague : for example, headaches, dizziness, irritability, insomnia, depression, poor concentration, apathy, forgetfulness, weight loss and self-neglect. Typically these are soon followed by involuntary movements (myoclonic jerks) and dementia, and other less constant findings include extrapyramidal, pyramidal, cerebellar, sensory and lower motor neuron signs (324-327). Classically the electroencephalogram shows diffuse slowing with periodic bursts of high-voltage slow waves. Death usually occurs from intercurrent infections between 4 months and 2 years after onset of the symptoms.

In their series of patients, Brown et al (326) found that the evolution of the disease was gradual in most cases, but in about a fifth, the onset was sudden. The mean illness duration was 8.5 months but this was heavily

influenced by the few cases which lasted from 2 to 10 years. In many patients who had a sudden onset, the course of the disease was rapid, with death occurring within 1 to 2 months.

The neuropathological changes of CJD resemble kuru, and occur mainly in the cortical and subcortical grey matter (324,325). They consist of diffuse neuronal loss, astroglial and sometimes microglial hypertrophy and hyperplasia, and neuronal vacuolation which leads to status spongiosus. Amyloid plaques showing some similarities to those seen in kuru, scrapie and Alzheimer's disease, are also found in CJD brains particularly in the cerebellum, in a minority of cases (328,329). Traub et al (312) for example, reported that 12.7% of 126 patients with CJD had such plaques.

Daniel (330) classifies CJD into 4 major types based on the distribution of lesions : the Jakob, Heidenhain, diffuse and ataxic forms.

In the Jakob type dementia is associated with painful sensations, spasticity of the limbs, and sometimes muscular atrophy. Although usually present throughout the cortex, the brunt of the degeneration of nerve cells falls most heavily on the cortical regions adjacent to the central sulcus, in the corpus striatum, thalamus and motor nuclei of the brain stem and spinal cord.

In the Heidenhain type, the pathological changes are most severe in the occipital region of the cortex, and changes are often also seen in the striatum and cerebellar

cortex. This type is associated with disturbances of vision, sensation, ataxia and dementia.

Nerve cell degeneration and astrocytic proliferation are widespread throughout the cortex, basal ganglia, thalamus, mid brain, cerebellum and spinal cord in the diffuse type, and the majority of patients with CJD fall into this group.

The cerebellum is the most severely affected part of the brain in the ataxic form, and ataxia is the most obvious clinical feature.

However there are some patients who show features of each group, and cannot be easily classified.

Klatzo et al (311) pointed out the close similarity of the pathological lesions observed in the brains of patients with kuru and CJD, and attempts at transmission of the disease were soon made following the success with kuru. In 1968 Gibbs and his associates (331) reported that biopsy material taken from the brain of a patient with CJD induced a similar fatal subacute spongiform encephalopathy in a chimpanzee 13 months after inoculation.

Since then, CJD has been successfully transmitted to chimpanzees, monkeys, cats, guinea pigs, hamsters and mice (312,332,316,318). The lymph nodes, liver, kidney, spleen, lung, cornea and cerebrospinal fluid of patients with CJD have been shown to harbour the virus in low titres, but the highest titres are found in the brain and spinal cord (318). The disease has been induced in some chimpanzees and monkeys inoculated peripherally by the subcutaneous, intraperitoneal, intramuscular and

intravenous routes, as well as the intracerebral route (318).

CJD is a rare disease occurring in under 1 per million population (333-335,339). Genetic factors are suggested by the familial clustering of disease (334-339), and the increased incidence of the disease in Libyan-born Jews (340). In a worldwide epidemiological study of 1435 patients with CJD, Masters and his colleagues (335) found that 15% of the cases were of the familial type. However a common source of exposure to the virus or vertical transmission of the agent, are alternative explanations for this apparent familial and racial clustering.

Brown et al (341) examined sera from CJD patients and chimpanzees for antibody titres to a number of known viruses including CMV, but no consistent pattern was found.

The question of the transmission of CJD remains unanswered. Some epidemiological surveys of CJD have failed to find any spatial or temporal clustering of cases, although other reports have suggested spatial clustering (333-335,342,343). Grabow et al (319) reported the case of a man developing CJD only 10 weeks after visiting Papua, New Guinea, implying that kuru and CJD may share a common pathogenic agent, and conjugal occurrence of the disease has been reported twice (344). CJD has been accidentally transmitted from human to human via corneal transplantation (345), and stereotactic intracerebral electrodes (346). There is also the possibility of

accidental transmission of CJD to a neurosurgeon who died of the disease (312).

Scrapie causes a disease indistinguishable from CJD in several species of monkeys. This has led to the suggestion that CJD could be transmitted through eating incompletely cooked scrapie-infected hog brains (347) and sheep's eyes (348) although the evidence for this is scanty, and the worldwide pattern of scrapie does not correlate with the distribution of CJD (335). Transmissibility of CJD to the domestic cat raises the possibility of an animal reservoir of infection (312), and a tenuous association of the disease with the keeping of ferrets has been reported (349). Previous surgery, particularly neurosurgery, and preexisting neurological disease, may increase the risk of developing CJD (335).

As kuru and CJD have clinical, pathological, and viral similarities, Traub and his colleagues (312) speculate that the two diseases may be caused by different strains of similar viruses. CJD has been observed in combination with a wide variety of other chronic neurological disorders (335) and in AD (329). Traub et al (312) postulate that CJD might be due to an opportunistic infection, which results from the invasion or perhaps reactivation of a latent virus, in a brain already diseased by a slowly progressive degenerative process.

VIRUSES AND ALZHEIMER'S
DISEASE

VIRUSES AND ALZHEIMER'S DISEASE:

Although there is little direct evidence at present to support the involvement of viruses in the aetiology of AD, their implication in other forms of dementia does suggest such a possibility. In fact, there are some histological and biochemical features of AD which are compatible with a viral aetiology.

Neurofibrillary tangles have been reported in diseases such as SSPE, herpes and rabies infections, and CJD, in which a viral aetiology has been confirmed (350). De Boni and Crapper (351,352) reported that paired helical filaments (PHF) morphologically resembling neurofibrillary tangles, are induced in cultured human foetal cortical neurons after exposure to an extract prepared from brain affected with Alzheimer's disease, and postulated that the PHF are a response of human neurons to an infectious agent.

Amyloid plaques with some similarities to those seen in AD, are sometimes found in CJD (312,328,329) and kuru (312), and similar amyloid plaques have also been detected in the brains of mice inoculated with some strains of scrapie (353,354), frequently in association with blood vessels.

The enzyme choline acetyltransferase which is reduced in the brains of patients with AD, is also reduced in murine scrapie (355).

The presence of nuclear bodies, a frequent

neuropathological feature of virus infection, has also been reported in the brain of a patient with AD (356), although this finding has yet to be confirmed.

However attempts to implicate viruses in the aetiology of AD by transmission of the disease to experimental animals has met with only limited success. Goudsmit and his colleagues (357) failed to transmit the disease to primates from 52 patients with AD, although a spongiform encephalopathy was transmitted in 2 cases. Although the possibility of laboratory contamination cannot be ruled out, this suggests that agents which produce spongiform encephalopathy in primates may be harboured in the brains of some patients with AD.

This evidence is admittedly circumstantial, but does raise the possibility that infectious agents, perhaps conventional viruses acting in concert with an unconventional agent or a disordered immune system, could play an aetiological role in AD. The brain is especially vulnerable to viral damage, because of its high energy requirements, low rate of DNA synthesis, extensive neuronal connections, and the inability of neurons to regenerate (238,358).

The herpes group of viruses, particularly herpes simplex virus (HSV), varicella zoster virus (VZV) and cytomegaloviruses (CMV), have properties which make them potential candidates for slow virus infection of the CNS (359,360,240,361).

Infection with these viruses are common, and most of

the population have detectable serum antibodies against them by middle age. They are neurotropic, and can persist within the nervous system in a latent form without overt disease for many years after primary infection. However reactivation of the virus from a latent state can occur despite the presence of circulating antibodies and disease ensue. For example, VZV persists in a latent state in sensory ganglia, following chicken pox in childhood, but can undergo reactivation, replication and transportation down the sensory ganglia many years later to produce the skin lesions of herpes zoster (359,360). Similarly some cases of herpes simplex encephalitis which can cause permanent memory impairment (362,363) may result from reactivation of the HSV from a latent state within the nervous system (240,361). Despite these characteristics, few studies of the possible role of conventional viruses in AD have been made.

Libikova and colleagues (364,365) found increased serum and CSF antibody titres to HSV 1 in demented patients compared with controls, and isolated the virus from the CSF of two demented. Lycke Norrby and Roos, (366) also reported an increased incidence of HSV antibodies in a group of senile and multi-infarct demented. However Lord et al (367) failed to confirm these findings, but found significantly higher levels of serum antibodies to adenovirus in demented patients than controls. Unfortunately as no attempts were made to differentiate AD from multi-infarct dementia, no firm conclusions can be

drawn from these serological surveys about the possible role of conventional viruses in AD. Edinburgh workers (368) measured serum antibodies to a number of viral and bacterial antigens in 14 patients with PDAT, but found no obvious associations. However Sequiera et al (369), using nucleic acid hybridization techniques, detected HSV 1 genome in the brains of three elderly patients, two of whom suffered from SDAT.

Lycke, Norrby and Roos (366) found a significantly higher incidence of CMV antibodies in patients with AD and multi-infarct dementia, compared with a control group, but concluded that differences in the standards of hygiene and age between the demented and controls may have been partly responsible. However, apart from the small Edinburgh study (368), there have been no attempts to replicate these findings.

The increasing prevalence of CMV antibodies with advancing age has prompted Weller (370) to ask in relation to this virus, if we should:

"not look for the visceral counterpart of the neurocutaneous lesions of zoster, perhaps manifested by transient fever, hepatitis, pneumonitis, nervous system involvement, or less overt symptoms?"

One of the purposes of this study is to examine the possible role of the CMV, whose properties are reviewed in the next section, in the aetiology of Alzheimer's disease.

THE CYTOMEGALOVIRUSES

THE CYTOMEGALOVIRUSES:

The cytomegaloviruses (CMV) are double-stranded DNA viruses which belong to the herpes-virus group (370-380). They were first isolated in 1956, and derive their name from their ability to produce large cells (cytomegalic cells) containing intranuclear and cytoplasmic inclusion bodies in epithelial cells. They also produce a specific cytopathic effect in tissue culture.

Human strains of CMV differ in antigenic structure, but it is unclear if they vary in their pathogenic potential. All strains share common group-specific complement-fixing (CF) antigens (381).

CMV infection is common (376, 380-382), and occurs predominantly in infancy and early adult life, when it is usually subclinical (374,375). About 50-60% of the population have detectable CMV antibodies by the age of 30, although this figure is higher in populations who live in poor, unhygienic and overcrowded conditions (376, 380-382). The persistence of antibodies in the adult population suggests that following primary infection, the CMV persist in a latent form inside cells without the production of new infectious virus. However the latent virus can undergo reactivation much later to produce new infectious virus with resulting clinical disease (374).

The prevalence of CMV antibodies is increased in old age (376,380,382,366), but it is not clear if this is the consequence of acquiring new infections or reactivation of latent infections following the age-related decline in

cell-mediated immunity.

The CMV are not highly contagious, and close and prolonged contact is necessary for infection (373,374). The commonest route of infection in adolescents and young adults is by direct oral contact, but spread may also occur through contact with infected urine, faeces, and breast milk, and perhaps through sexual intercourse (373, 374).

Humoral immunity as determined by circulating antibody to CMV is normal in CMV infections, but cell-mediated immune responses to the virus are impaired (383-394).

Most CMV infections are either asymptomatic or mild in nature, but some are associated with clinical disease:

1. Congenital CMV infections:

The incidence of primary CMV infection in pregnant women is about 1-2%, and in about half these cases, babies are born infected, and excreting CMV (373,374,395).

Most babies with congenital CMV infection are asymptomatic, or have relatively mild features, such as prematurity, low birth weight, failure to thrive, jaundice, hepatosplenomegaly, purpura and pneumonia (373,374,395, 398). However about 10% of these ultimately develop mental retardation, visual and hearing defects and congenital heart disease (373,374,395,398,399-401). Severe congenital CMV infections can result in intrauterine death or cytomegalic inclusion disease.

Cytomegalic inclusion disease (374,376,380,396,397)

presents shortly after birth with a variety of clinical features including jaundice, hepatosplenomegaly, thrombocytopenic purpura, pneumonia, chorioretinitis, haemolytic anaemia, congenital heart defects, microcephaly, encephalitis, hydrocephalus, and intracranial calcification. Most survivors have severe brain damage, resulting in psychomotor and mental retardation, epilepsy, palsies, blindness and deafness. Much of the tissue damage results from the direct cytopathogenic effect of the virus but immune abnormalities may also be contributory.

2. Acquired infection in children and adults:

CMV infections have been implicated in atypical Paul-Bunnell-negative glandular fever (402-405), hepatitis (375,386,406-409), pneumonitis (410), pericarditis (407), autoimmune haemolytic anaemia (411,412) and the post-perfusion syndrome (413-417). CMV infection, either newly acquired or following reactivation, can be a serious and sometimes fatal complication of intensive immunosuppressive therapy in conditions such as leukaemia (418-422), Hodgkin's disease (418,421), and renal transplantation (423-431).

CMV infections have also been reported in association with a variety of neurological disorders involving the peripheral and central nervous systems.

They are a well established cause of polyneuritis (Guillain-Barre syndrome) (432-437). Dowling, Menonna and Cook (436) found serological evidence of recent CMV

infection in 30 out of 92 patients with polyneuritis. Schmitz and Enders (435) reported that CMV infection preceded the onset of polyneuritis in 10 out of 94 patients, and Duchowny, Caplan and Siber (437) described a patient in whom systemic CMV infection was complicated by a brachial plexus neuropathy.

CMV have also been implicated in acute and chronic encephalitis (437-441).

CMV encephalitis is rare in immunologically normal adults. Philips et al (441) reported the occurrence of acute CMV encephalitis in a man and a woman with normal immune systems. Viral cultures of urine, and cerebrospinal fluid (CSF) yielded CMV in both cases as did a brain biopsy specimen in the woman. However serum CMV CF antibody only reached a titre of 1:16 during and after the illness in the man, and no antibody could be detected in the serum of the woman.

Duchowny, Caplan and Siber (437) also reported the case of a 30 year old previously healthy doctor who had a relapsing chronic CMV encephalitis with a clinical history extending over several years. Yanagisawa, Toyokura and Shiraki (442) described a 51 year old housewife with double encephalitis due to herpes simplex virus (HSV) and CMV. They postulated that in the course of a necrotizing - encephalitis by HSV, the debilitated state and treatment with steroids and antibiotics promoted a CMV infection in the brain.

CMV encephalitis is usually associated with immunosuppression. Schneck (443) found evidence of CMV

encephalitis in 12 out of 34 immunosuppressed patients who had died following kidney transplantation. Glial nodules, predominantly in gray matter, and consisting mainly of microglial cells with occasional astrocytes were found in 11 cases. Inclusion bodies and cell changes compatible with CMV infection were noted in 2 brains. Dorfman (440) described the neuropathological findings in the brains of 4 adults with CMV encephalitis, three of whom had undergone renal transplants and immunosuppressive treatment. All 4 patients had widespread glial nodule encephalitis thus confirming Schneck's findings. Two of the brains also contained cytomegalic cells with prominent intranuclear inclusions.

There have been few attempts to study the effects of CMV on the nervous system of animals. Brain damage resembling that found in human cytomegalic inclusion disease has been induced in suckling mice by intracerebral inoculation with mouse CMV (444). Wroblewska et al (445) reported the development of an acute demyelinating disease in a chimpanzee more than 3 years after intracerebral inoculation at birth of brain cell cultures derived from a patient with multiple sclerosis. A strain of CMV was isolated from the left frontal lobe of the animal but it is possible that it was transmitted from other chimpanzees and not from the inoculated MS brain.

THE HLA SYSTEM

THE HLA SYSTEM:

The HLA* or major histocompatibility system of man consists of a group of closely linked genes located on the short arm of chromosome 6, at 4 loci designated A, B, C and D (figure I), which appear to play a central role in the regulation of important biological functions (446-450, 452, 453, 455, 457).

Although many gene alleles are possible, only one allele is found at each locus at any one time. At present 20 alleles at the A, 33 at the B, 6 at the C, and 11 at the D loci are recognized, although more probably await recognition at the C and D loci (459).

The products of these genes are called HLA antigens. The HLA-A, -B and -C antigens are glycoprotein structures found on the surfaces of all nucleated cells of the body including leucocytes and T and B lymphocytes, and comprise 1-2% of cell membranes (460-462). They develop in the foetus at about 6 weeks and persist throughout life. They consist of two polypeptide chains: a light chain with a molecular weight of 12,000 daltons called β_2 - microglobulin, coded for by a gene on chromosome 15, which is non-covalently linked to a heavy chain with a molecular weight of 43,000. The heavy chain bears the antigenic

*H for human; L for leucocyte, the first cells shown to carry antigens of this system; and A for the first locus identified.

Figure 1 - Loci composing the human HLA region(220)



Brackets and arrow indicate that the order of loci is not known.

Loci designated by the same pattern belong to the same class.



Class I loci.



Class II loci. The number of class II loci is uncertain. The designation of class II loci has not been agreed upon.



Class III loci.



A possible fourth class of loci related to class I loci.

GLO

An enzyme-coding locus.

determinants which confer HLA specificities on the molecule by variation of its aminoacid sequences (460-462).

The combination of HLA antigens on each chromosome is called a haplotype. Therefore each person has two haplotypes, and millions of HLA antigen combinations are possible (463). Both parents contribute one haplotype each to their offspring resulting in four possible haplotype combinations (462).

The HLA -A, -B, and -C antigens are detected serologically by a microlymphocytotoxic technique using lymphocyte suspensions prepared from peripheral blood (455,460,462). The lymphocyte suspensions are delivered by micropipette to wells containing standardized antisera to each specificity, and complement is added. The antibodies used do not occur naturally, but are found in the sera of people such as pregnant women, blood transfusion and transplant recipients, who have been exposed to foreign HLA antigens. Lymphocytes bearing a particular HLA specificity are killed when exposed to its specific antibody, the number of dead cells being estimated visually or automatically using a fluorescent dye or radioactive technique. Individuals have a maximum of two antigens at each HLA locus, although sometimes only one is detectable because they are homozygous or have a determinant as yet unidentified.

The D-locus antigens are identified using the more complicated mixed lymphocyte culture (MLC) technique (455,

464). More recently HLA -DR (D-related) antigens which appear to be closely related to the D-locus antigens, have been defined serologically by lymphocytotoxicity in B-lymphocyte enriched suspensions (461,465). Typing for HLA-C and DR antigens is not done routinely at present because antisera are not widely available. Similarly as the MLC is a complicated technique, the identification of D-locus antigens is restricted to a few laboratories.

Much of our understanding of the importance of the HLA system has been derived from the study of the H-2 complex (453,457,466), the equivalent genetic region in the mouse.

Products of the genes within the H-2 system are known to participate in immune responses to viruses, code for major transplantation antigens which are involved in graft rejection, mediate protection against intracellular bacterial infections and regulate certain cellular-immune responsiveness assessed by antibody production (454). The H-2 system has also been implicated in the genetic control of lifespan in congenic mice (470).

Genes within the HLA region also appear to play a central role in the control of immune responses in man (447, 448,450,453,457,460,463). In particular Ir (immune response) genes have been defined and arguments put forward that these may be identical with the Ia genes in the mouse

and hence with the homologous DR antigens in man.

The Ir genes are thought to control T-cell responses to antigens by influencing the proliferation and function of helper-T cells, suppressor-T cells, and cytotoxic-T cells, and also T cell -B cell, and T cell-macrophage interactions (454,456). Recent findings suggest that the major histocompatibility system also plays an important role in cell-cell communication and differentiation of non-lymphoid as well as lymphoid cells, as it has been implicated in phenomena such as intercellular adhesion, contact inhibition, lymphocyte recirculation and stem cell growth (458).

Although not coding for antibody molecules, the HLA region probably influences their production through the interaction of helper-T cells with B cells.

HLA genes also control the synthesis of some components of the complement system (C2, C4, factor B) (453,457,468), and HLA antigens play an important role in the rejection of organ transplants (455,469).

A number of human diseases have been found to be associated with particular HLA antigens (449,451,471-473), although the reasons for such associations are unclear. Dausset (474) has described the features which commonly characterise these HLA-associated disorders:

- 1) They are of unknown aetiology.
- 2) No causal agents have been identified, although in some cases, viruses have been suspected.
- 3) They are often hereditary, but with weak

polyfactorial and probably polygenic penetrance.

4) Disease susceptibility is transmitted within families, but the segregation does not follow simple mendelian laws.

5) Frequently there are immunological abnormalities such as cellular infiltrations in the tissue lesions, circulating antiviral antibodies, or autoantibodies.

6) The course of the diseases are usually subacute or chronic.

7) The diseases are seldom manifested before adulthood, and there is little impact on reproduction or selection.

Most HLA associations are with the B-locus antigens, but some diseases show even stronger associations with the HLA-D/DR antigens. This is because of linkage disequilibrium (463,475), the tendency for certain HLA antigens at different loci to occur together more frequently on the same haplotype than expected by chance, the stronger association occurring the closer the particular HLA locus is to that for the disease susceptibility gene.

Two main approaches are used in HLA studies (473):

Population studies:

Population studies involve the comparison of the frequencies of HLA antigens in a group of patients with a particular disease, with the corresponding frequencies in a group of healthy unrelated controls of the same ethnic origin. A positive association between a disease and a particular HLA antigen occurs if the frequency of the antigen is increased in the patients compared with the controls.

The relative risk (RR) - the number of times more often the disorder develops in persons possessing this antigen as compared to those in whom it is absent - is always greater than one in positive associations, and gives some measure of the biological significance of the association.

Population studies have successfully identified a number of HLA-associated diseases, including ankylosing spondylitis, Reiter's disease, certain sero-negative arthropathies, psoriatic arthritis and acute anterior uveitis which are all associated with HLA-B27 (451,472, 473), juvenile-onset diabetes with HLA-B8, B15, Cw3, Dw3 and Dw4 (451,472,473), and multiple sclerosis with A3, B7 and Dw2 (451,472,473).

The relative risk for most diseases is under 10, but people with HLA-B27 have a relative risk of about 80 of developing ankylosing spondylitis (472).

Family studies:

If more than one member of a family is affected by the disease family members can be HLA typed to see if affected relatives share haplotypes more often than expected. Although not commonly used, family studies allow the possibility of detection of linkage of disease genes to HLA genes even if an association is not found in the general population.

There are some grounds to expect that AD might be associated with an HLA antigen, as the disease shows many of the typical features of HLA-associated disorders. A positive association would improve our understanding of AD by confirming the involvement of genetic factors and helping to identify their nature. It might also help to clarify the relationship between PDAT and SDAT, and would be further evidence that immune factors may be implicated in the disease, as many of the HLA-associated disorders are thought to be immunologically mediated.

ABO AND RHESUS BLOOD

GROUPS AND DISEASE

ABO AND RHESUS BLOOD GROUPS AND DISEASE:

Many red blood cell groups have been defined, but the ABO and rhesus blood groups are clinically the most important, and have been most frequently used as genetic markers in the search for blood group and disease associations (476).

ABO BLOOD GROUPS:

In man, 4 major ABO blood groups are recognized (477, 478) - A, B, AB and O - which are determined by the presence or absence of the antigens A, B and H on red blood cells. These antigens are also found in most secretions and tissues of the body except the central nervous system, and are controlled by 3 allelomorphic genes A, B and O located on chromosome 9 (479).

ABO genes are thought to control the synthesis of transferase enzymes which catalyze the formation of polysaccharides, which combine with lipids to produce cell surface glycolipids (478). It is thought that a basic substance H, which is present in all red cells, is mostly converted into group A and B antigens by their respective transferases which are coded for by the genes A and B. The O gene is considered to be an amorph or silent gene, with no detectable effect on the H substance.

ABO BLOOD GROUPS AND DISEASE:

The ABO blood group antibodies have been directly implicated in haemolytic disease of the newborn (480) and haemolytic transfusion reactions (481). However there is also evidence to suggest that persons with different ABO blood groups vary in their susceptibility to certain diseases (476,482-484), although the increased risk is generally small.

In ABO and rhesus blood group and disease association studies, patients with a particular disease are blood grouped, and the frequencies of the different groups are then compared with those among a control group of people unaffected by the disease. An association is suggested if the disease is increased significantly in patients of a particular blood group. The most striking findings, which have been confirmed in many studies, are the increased frequencies of blood group A and O in patients with gastric carcinoma, and duodenal and gastric ulceration, respectively (482,483,485-487). The increased risk of persons developing these diseases with the appropriate blood group varies from 1.2 - 1.4 times. Clarke et al (488) found an even closer association between the hereditary character of nonsecretion of ABH substances in the saliva, and duodenal ulceration.

Associations of blood group A with coronary thrombosis, pernicious anaemia, smallpox and malaria have also been claimed (476).

Unfortunately the accuracy of many of the positive findings of ABO blood group and disease association studies is questionable. Some of the significant results are derived from single studies, and the data obtained from multiple studies often show very large discrepancies.

RHESUS BLOOD GROUPS:

The rhesus (Rh) blood group system is thought to be determined by 3 pairs of closely linked genes (C, D and E, and allelic forms c, d and e) (489), which are inherited as a group on chromosome 1 (479). A combination of these three genes which code for the Rh antigens (C and c, D and d, and E and e) found on red blood cells, are inherited from each parent (489).

Thirty six genotypes are possible, and on testing with the appropriate Rh antisera, the population can be subdivided into 15 Rh phenotypes (489). However the D antigen is clinically the most important of the Rh system because of its greater capacity to stimulate antibody formation.

In Rh typing, only anti-D serum is usually used to classify persons Rh negative or Rh positive. Rh negatives are homozygous for the d gene, and Rh positives are either homozygous DD, or heterozygous Dd.

Rh BLOOD GROUPS AND DISEASE:

Incompatibility with respect to the Rh antigens is an important cause of haemolytic disease of the newborn (480), and haemolytic transfusion reactions (481). Both disorders result from Rh antigen - antibody incompatibility.

However, the Rh blood groups, like the ABO blood groups, may influence susceptibility to some diseases in a

less direct way. Statistically significant increases of Rh negatives have been reported in typhoid and paratyphoid infections, mumps, infectious mononucleosis, viral meningitis, tuberculous infections of bones and joints and the genito-urinary system (476), and multiple sclerosis (490).

The reservations made about the accuracy of many of the positive findings of ABO blood group and disease association studies apply equally to rhesus blood group and disease association studies.

METHODS

THE STUDYMETHODSPATIENTSa) Case Finding:

The study patients were selected from unrelated inpatients at five psychiatric hospitals in the Yorkshire region.

Potential study patients were identified following consultation with medical and nursing staff. Their case notes were examined, and any patients who lacked details of past medical and psychiatric histories, or the results of appropriate physical examination and laboratory investigations, were excluded.

For consideration, patients had to fulfil the following criteria.

b) Selection criteria:Senile dementia of the Alzheimer type:

1. A history of dementia with an insidious onset after the age of 64, and a slowly progressive impairment of memory, intellect and personality.

2. No previous psychiatric or occupational history

which might account for the dementia - e.g. alcoholism, drug abuse, drug overdoses, gassing, boxing.

3. No family history of other dementias such as Huntington's chorea.

4. No previous history of significant physical disease which could have caused dementia - e.g. epilepsy, strokes, brain tumours, hypertension, peripheral vascular disease, diabetes.

5. Clear evidence of dementia on clinical examination.

6. Absence of significant cardiovascular disease or focal neurological signs.

7. Essentially normal results in the following investigations:

Haemoglobin, and ESR,
Urea and electrolytes,
Liver function tests,
Fasting blood glucose,
Thyroid function tests,
Serum vitamin B₁₂ and folate,
Serum WR or VDRL,
Urinanalysis,
Chest and skull X-ray,
ECG.

Some patients had been further investigated with EEG's, air encephalography, brain and CAT scans, and the results were compatible with a diagnosis of Alzheimer's disease.

Presenile dementia of the Alzheimer type:

1. A history of dementia, starting insidiously before the age of 65, with early memory impairment and disorientation, parietal lobe features such as apraxia and aphasia, and progressive impairment of memory, intellect and personality.

2. No previous psychiatric or occupational history which might account for the dementia - e.g. alcoholism, drug abuse, drug overdoses, gassing, boxing.

3. No family history of other dementias such as Huntington's chorea.

4. No previous history of significant physical disease which could have caused dementia - e.g. epilepsy, strokes, brain tumours, hypertension, peripheral vascular disease, diabetes.

5. Clear evidence of dementia on clinical examination.

6. Absence of significant cardiovascular disease or focal neurological signs.

7. Essentially normal results in the following investigations:

Haemoglobin, and ESR,
Urea and electrolytes,
Liver function tests,
Fasting blood glucose,
Thyroid function tests,
Serum vitamin B₁₂ and folate,

Serum WR or VDRL,
Urinalysis,
Chest and skull X-ray,
ECG.

c) Assessment of patients:

Patients who satisfied these criteria were interviewed, and their mental and cognitive state assessed. Cognitive abilities were formally tested with the Mental Test Score (83), although some patients were too demented to co-operate.

This was supplemented by information from the nursing staff about their behavioural functioning such as ability to dress, feed and communicate.

Patients who showed clear evidence of dementia were then physically examined to confirm the absence of significant cardiovascular disease or focal neurological signs. Any outstanding laboratory investigations were completed, and data for the study recorded from the case notes of the patients.

A total of 125 patients who satisfied the criteria for Alzheimer's disease were selected for study. However a postmortem on a male patient in the SDAT group failed to confirm the clinical diagnosis and he was excluded from the study.

The characteristics of the 124 patients are shown in Tables 1 and 2.

A 15 year old girl with a clinical diagnosis of Creutzfeldt-Jakob disease later confirmed at postmortem, was also studied, but this case will be described separately.

d) Identification of secondary cases of AD:

The case notes rarely contained details about the occurrence of AD in other family members, and field work was necessary to obtain this information.

The next of kin of 10 HLA-B15 positive and 10 HLA-B15 negative patients matched for sex and age at onset of AD, and also 14 other HLA-B15 positive patients, with no recorded family history of AD in their case notes, were interviewed at home when a physical and psychiatric history of the first degree relatives was compiled.

A diagnosis of AD was made in the relatives if a history of progressive dementia in the absence of strokes or other significant physical disease was elicited.

However this study was not comprehensive, as in some cases, contact had been lost with individual family members, or information about deceased relatives was lacking.

The two relatives affected with dementia who were still alive, were traced, HLA-typed and ABO and Rh blood grouped.

e) Follow up of patients:

In order to determine if HLA antigens influence life expectancy in AD, patients were revisited about 18 months after completion of the serological studies. The case notes of those who had died were traced from medical records, and the date of death recorded.

SEROLOGYa) Specimens:

About 30 mls of venous blood was collected from each patient in a lithium heparin and 2 plain glass tubes. These specimens were taken to the Leeds Regional Blood Transfusion Centre for blood grouping and HLA typing, and to the Leeds Public Health Laboratory for estimation of CMV antibody titres.

b) HLA Typing:

One hundred and twenty five patients were tested for 20 of the main HLA-A and -B group antigens using the National Institutes of Health (NIH) lymphocytotoxicity technique (462) (Appendix 1).

All the HLA specificities were determined using at

least 3 well-defined antisera (Appendix 2).

c) ABO and Rh Blood Grouping:

The ABO and Rh (D) blood groups of 125 patients were determined using standard agglutination techniques (491).

d) CMV Antibodies:

Sera were stored at -20°C until tested, and examined for antibodies to the CMV complement-fixing (CF) antigens. The complement fixation test used was a modification of that described by Bradstreet and Taylor (492), and was performed by a microtitration technique. Serum dilutions started at 1 in 16.

CMV antibody titres were determined in only 113 of the 125 patients, none of whom were on steroids or immuno-suppressive drugs. The virus study was started later than the blood group studies, and the other patients had either died or been transferred elsewhere before sera could be taken for CMV antibody estimations.

POST-MORTEM STUDIES

AD can be diagnosed with certainty only at post-mortem. Therefore post-mortems were requested on all the

study patients who died at High Royds Hospital, in order to obtain some measure of the accuracy of clinical diagnosis, and also to allow for biopsy specimens of the brain, liver and kidney to be taken for viral studies in a few cases.

VIRUS ISOLATION ATTEMPTS

Samples from the brain, liver and kidney biopsies of 8 patients were inoculated into human embryonic lung fibroblasts. These cell cultures were maintained in Eagle's basal medium with foetal calf serum, 100 units of penicillin/ml and 100 micrograms of streptomycin/ml at 37° C for one month. During this time they were examined at regular intervals for cytopathic effect (CPE) caused by cytomegalovirus. Cultures showing no CPE after one month were regarded as negative for cytomegalovirus.

CONTROLS

a) HLA ANTIGENS:

The control group for the HLA antigen study comprised a group of 458 normal Leeds blood donors and healthy hospital staff.

b) ABO AND Rh BLOOD GROUPS:

The controls for the ABO and Rh blood group study consisted of a group of new blood donors drawn from approximately similar catchment areas as the patients, and were taken from the tables of Kopec (493,494). In addition the ABO and Rh blood group frequencies of 196 of the 458 Leeds HLA antigen controls were known and used as controls.

c) CMV ANTIBODY STUDY:

Thirty-nine non-demented patients suffering from functional psychiatric disorders (mainly schizophrenia) who had been inpatients at High Royds Hospital for many years, and who were of a similar age to the study patients, were selected to act as controls for the CMV antibody study after being interviewed by the writer. Their characteristics are shown in Table 6. The HLA antigen phenotypes were not determined in this group.

STATISTICAL PROCEDURES

Differences in the frequencies of HLA antigens and ABO and Rh blood groups in the patients and controls were tested with a 2 x 2 chi-square test with Yates' correction when indicated. When multiple comparisons are being made, there is an increased chance that a statistically "significant" probability value will be observed in respect of one of the comparisons when in fact there is no real association. Therefore the probabilities obtained in the HLA study were multiplied by 20, the number of antigens tested, to give a corrected P value.

Patients were subdivided according to the presence or absence of HLA-B15, an HLA antigen found to be significantly associated with AD in this study, and analyzed in relation to sex, age at onset of disease, length of illness, and family history of dementia, to determine if these clinical characteristics influenced the strength of the association. Statistical comparisons between these two groups were made using the Student's t test and chi-square test. In the family studies, only relatives thought to be at risk of developing AD - i.e. those over 44 years of age, the age at which the youngest patient developed AD in this study - were included in the analysis of data.

The ABO, Rh and HLA data obtained from similar studies performed at different centres were combined with data from this study using Woolf's method of statistical

analysis (521,522). Woolf's method allows a more accurate assessment of the relative risk and statistical significance of any association between AD and these genetic markers to be made, and also tests for heterogeneity between centres.

The prevalence of CMV antibodies in the patients and the controls were compared using the chi-square test.

The data on CMV antibody titres were transformed to logarithms to the base 10, and the differences in the mean titres of the patients and controls were determined using the Student's t test. Titres that were less than 16 were taken to be 8 for the purposes of statistical analysis.

THE ROLES OF THE WORKERS ENGAGED IN THE STUDY

In the study, the case finding, the psychiatric and physical assessment of patients, the collection of serological specimens and their delivery to the laboratories, the field work with relatives and the follow-up of patients, were made by the writer.

The HLA typing and blood grouping, and the virology studies, were performed by the technicians of the Leeds Regional Blood Transfusion Centre, and the Leeds Public Health Laboratory, respectively. The postmortems were performed by pathologists from the Department of Neuropathology, Leeds University.

RESULTS

THE RESULTS:

1) General information on the patients studied:

Tables 1 and 2 show the sex, age at onset of the disease, ABO and Rh blood group, HLA type, and CMV antibody titre of the patients in the PDAT and SDAT groups respectively, and also the length of the disease and the histological diagnosis if known, of the dead patients in these groups.

Table 3 shows some of the data from tables 1 and 2 in summary form, and also the mean age at onset in the presenile and senile groups. There is an excess of senile patients and women in the study, which reflects the greater prevalence of the senile form of AD and also the female preponderance found in this disorder.

Tables 4 and 5 show the mean age at onset in the male and female presenile and senile patients respectively. No significant differences are found between the men and women.

2) General characteristics of the patients and controls for the CMV study:

Table 6 compares some of the characteristics of the patients and controls for the CMV study. There are more

men than women in the control group, but the mean ages of the patients and the controls at the time of study do not differ significantly.

3) ABO blood group frequencies in the patients and controls:

Table 7 shows the frequencies of the ABO blood groups in the patients and controls. No significant differences in ABO blood group distributions are found between the patients and controls.

4) Rhesus blood group frequencies in the patients and controls:

Table 8 shows the frequencies of the Rh blood groups in the patients and controls. The Rh blood group frequencies in the PDAT group do not differ significantly from the controls.

However significantly more of the senile patients are Rh negative compared with the controls of Kopec (494) ($X^2 = 6.442$, $df = 1$, $P = 0.011$), or the Leeds Blood Transfusion (BTS) controls ($X^2 = 5.235$, $df = 1$, $P = 0.022$).

No significant differences are found in Rh blood group distribution between the combined group of PDAT and SDAT patients and the controls of Kopec (494) ($\chi^2 = 4.807$, $df = 2$, $0.1 > P > 0.05$), or the Leeds BTS controls ($\chi^2 = 3.777$, $df = 2$, $0.5 > P > 0.1$).

5) HLA antigen frequencies in the patients and controls:

The HLA antigen data for the PDAT, SDAT and combined groups of patients shown in Tables 1 and 2, are summarised in Tables 9, 10 and 11 respectively.

Table 9 shows that the frequency of HLA-B15 is significantly increased in the presenile group (32.43% v 13.75%, $\chi^2 = 9.224$, $df = 1$, $P = 0.0024$, corrected $P = 0.048$), compared with the controls. The frequencies of the other antigens do not differ significantly.

Table 10 shows that the frequency of HLA-B15 is also significantly increased in the senile group (26.44% v 13.75%, $\chi^2 = 8.837$, $df = 1$, $P = 0.003$, corrected $P = 0.06$) compared with the controls. The other HLA antigen frequencies do not deviate significantly from the controls.

Table 11 shows that the increase in frequency of HLA-B15 remains significant when the presenile and senile

patients are combined (28.20% v 13.75%, $\chi^2 = 14.619$, $P < 0.001$, corrected $P < 0.02$), but no significant deviations are seen with the other HLA antigens.

6) HLA-B15 and clinical characteristics of the patients:

(i) Frequency of HLA-B15 in men and women:

Tables 9, 10 and 11 show that there are no significant differences between men and women in the frequency of HLA-B15, although more women than men have this antigen in the presenile group.

(ii) HLA-B15 and age at onset of AD:

Figure 2 shows the percentage of patients who are HLA-B15 positive in relation to their approximate age at onset of AD.

Tables 12 and 13 show the mean ages at onset of AD in the HLA-B15 positive and negative presenile and senile patients respectively. No significant differences are found between the HLA-B15 positives and negatives.

(iii) HLA-B15 and length of history of the disease in the deceased patients:

Tables 14 and 15 show the approximate length of

history of AD in the deceased PDAT and SDAT HLA-B15 positive and negative patients respectively. No significant differences are found between the HLA-B15 positives and negatives.

7) Family studies:

Table 16 shows the results of the family studies of the first degree relatives of 10 HLA-B15 positive and 10 HLA-B15 negative patients matched for sex and age at onset of disease. Table 17 shows the family histories of AD in a further 14 unmatched HLA-B15 positive patients.

9.7% of the relatives of the 34 patients studied had a history of AD, and cases of PDAT and SDAT were found to occur within the same families.

The frequency of AD in first degree relatives was significantly higher in the 24 B15 positive patients when they were compared with the 10 B15 negative patients (13% v 3.3%, $\chi^2 = 4.5924$, $P = 0.0324$). However no statistically significant difference in the frequency of family history of AD was observed when the 10 B15 positive and 10 B15 negative patients matched for sex and age at onset of disease were compared (8% v 3.3%, $\chi^2 = 1.2572$, $0.5 > P > 0.1$).

Patients numbers 69 and 62 had a similarly affected relative still alive, and the results of the HLA, ABO and

Rhesus blood group studies of these two secondary cases are also shown in Tables 16 and 17 respectively.

An unconfirmed history of leukaemia was reported in the relatives of patients numbers 50 and 104.

8) CMV antibody titres in the patients and controls:

Table 18 shows the number and percentage of patients and controls with CMV antibodies at different titres.

More patients than controls have antibody titres $\geq 1/16$, but the difference is not significant (85.84% v 76.92%, $X^2 = 1.678$, $df = 1$, $0.5 > P > 0.1$).

Table 19 shows the serum geometric mean antibody titres (\log_{10}) to CMV of the patients and controls. The mean titre is higher in the patients than the controls, but the difference is not significant.

Table 20 shows the CMV antibody titres of the HLA-B15 positive and negative patients. Significantly more HLA-B15 positive than negative patients have a titre $\geq 1/64$. (57.6% v 26.25%, $X^2 = 10.027$, $P = 0.0016$).

Table 21 shows the geometric mean CMV antibody titre of the patients in relation to HLA-B15. The mean titre is significantly higher in the HLA-B15 positives.

9) Post-mortem studies:

Post-mortems were obtained on 18 of the 46 patients who died at High Royds Hospital (tables 1 and 2).

Alzheimer's disease was confirmed in 14 patients, and mixed AD - multi-infarct dementia diagnosed in 2 further cases. In patient number 2, widespread autolytic changes due to delayed autopsy prevented a positive confirmation of the macroscopic diagnosis of AD. The histological diagnosis of multi-infarct dementia was made in patient number 125, and he was excluded from the study. Although consent was not obtained for a post-mortem on patient number 12, a cerebral biopsy had previously confirmed the clinical diagnosis of AD.

10) Virus Isolation Attempts:

Brain, liver and kidney biopsies from patients number 2, 6, 46, 56, 64, 77, 84 and 98 failed to induce any CPE in cell cultures.

Table 1(i). Characteristics of the presenile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|-----------------|---------------------------------|--|--------------------------------------|
| 1 L.M. | 52 | M | A | + | A2, B5 | 64 | 12 | |
| 2 M.H. | 53 | F | A | + | A2, B40 | < 16 | 6 | * |
| 3 C.O. | 64 | F | A | + | A1, B14, 15 | | 11 | AD |
| 4 E.S. | 63 | F | O | + | A1, 2, B8, 12 | 64 | | |
| 5 L.W. | 64 | F | A | + | A3, 9, B7 | < 16 | | |
| 6 J.N. | 51 | M | A | + | A9, 11, B12, 15 | 32 | 9 | AD |
| 7 M.D. | 63 | F | O | + | A1, 2, B27 | 16 | 11 | AD |
| 8 N.B. | 55 | F | O | + | A2, B5, 12 | 32 | 9 | |
| 9 A.B. | 63 | M | O | + | A1, 2, B13, 15 | < 16 | | |
| 10 M.S. | 49 | F | O | + | A1, B17, 35 | < 16 | 15 | AD |
| 11 W.L. | 61 | M | B | + | A2, 9, B7, 14 | | | |

Table 1(ii). Characteristics of the presenile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|--------------|---------------------------------|--|--------------------------------------|
| 12 F.S. | 55 | F | O | - | A2,3,B12,15 | 64 | 15 | AD** |
| 13 R.H. | 61 | M | A | + | A2,3,B7 | | 8 | AD |
| 14 J.G. | 53 | F | A | + | A1,2,B12,15 | 32 | | |
| 15 H.S. | 64 | F | O | - | A2,B12 | | | |
| 16 I.S. | 54 | F | O | + | A1,B12,15 | 64 | | |
| 17 W.M. | 47 | M | B | - | A28,B5,35 | 64 | 4 | |
| 18 J.M. | 62 | M | O | - | A10,11,B7,40 | 32 | 4 | |
| 19 C.A. | 60 | F | A | + | A1,2,B5,27 | 16 | | |
| 20 V.B. | 64 | F | O | + | A1,B8 | 128 | | |
| 21 E.B. | 64 | F | A | + | A1,B8,13 | 32 | | |
| 22 O.S. | 49 | F | O | + | A1,2,B15,35 | < 16 | | |

Table 1(iii). Characteristics of the presenile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|----------------|---------------------------------|--|--------------------------------------|
| 23 E.C. | 59 | M | O | + | A2, B7, 27 | 16 | | |
| 24 A.S. | 63 | M | A | + | A2, 3, B12 | < 16 | | |
| 25 C.O. | 56 | F | A | + | A1, B12, 35 | 16 | | |
| 26 I.F. | 53 | F | O | + | A2, 3, B7, 35 | 16 | | |
| 27 N.F. | 58 | F | A | + | A1, 2, B15, 40 | 32 | | |
| 28 J.Q. | 51 | M | O | - | A1, 11, B8, 14 | 32 | | |
| 29 W.C. | 60 | M | A | + | A1, B12, 17 | < 16 | | |
| 30 O.C. | 52 | F | A | + | A2, B15, 40 | 64 | 4 | |
| 31 B.W. | 60 | F | O | + | A1, 2, B8 | 32 | | |
| 32 M.O. | 45 | M | O | + | A2, B7, 12 | 32 | | |
| 33 D.L. | 55 | F | A | - | A3, 9, B7, 14 | 16 | | |

Table 1(iv). Characteristics of the presenile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|---------------|---------------------------------|--|--------------------------------------|
| 34 A.W. | 61 | M | A | + | A2, B15, 35 | 64 | | |
| 35 B.S. | 62 | F | O | + | A1, 2, B7, 35 | 32 | 4 | |
| 36 A.W. | 63 | F | A | - | A2, B15 | 16 | | |
| 37 M.R. | 50 | F | A | + | A1, B15, 40 | 32 | 11 | AD |

* Widespread autolytic changes due to delayed autopsy prevented a positive confirmation of the macroscopic diagnosis of AD.

** Histological diagnosis from brain biopsy.

Table 2(i). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|---------------|---------------------------------|--|--------------------------------------|
| 38 C.L. | 70 | F | A | + | A28,10,B14,35 | 64 | | |
| 39 M.M. | 78 | F | A | - | A1,2,B8,12 | | 6 | |
| 40 N.G. | 79 | F | A | + | A3,28,B35 | 64 | | |
| 41 D.S. | 81 | F | A | + | A2,3,B27,35 | | | |
| 42 E.S. | 69 | F | A | + | A3,9,B5 | 32 | | |
| 43 E.H. | 68 | F | AB | - | A2,3,B12 | 16 | 5 | AD |
| 44 G.O. | 71 | F | O | + | A3,9,B7,35 | 16 | | |
| 45 B.L. | 78 | M | O | + | A10,B15,35 | 64 | 4 | |
| 46 L.T. | 75 | F | A | + | A2,11,B12,17 | 32 | 5 | Mixed AD - multi-infarct dementia |
| 47 M.T. | 70 | F | A | + | A2,B18 | 64 | 9 | |

Table 2(ii). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|----------------|---------------------------------|--|--------------------------------------|
| 48 M.F. | 71 | F | A | + | A2, 10, B12 | 64 | | |
| 49 C.C. | 71 | F | O | + | A1, 9, B8, 35 | 128 | | |
| 50 H.H. | 77 | M | A | + | A1, 3, B15, 17 | | 4 | |
| 51 C.W. | 73 | F | O | + | A2, B12 | 16 | | |
| 52 R.A. | 70 | F | A | + | A2, 9, B27, 40 | < 16 | | |
| 53 W.W. | 78 | M | A | + | A2, 3, B7, 15 | 128 | 9 | |
| 54 G.D. | 71 | F | O | + | A1, 9, B8, 35 | < 16 | | |
| 55 C.C. | 72 | M | O | + | A2, 9, B12 | < 16 | | |
| 56 S.W. | 78 | M | O | + | A2, 9, B7, 27 | 16 | 5 | AD |
| 57 S.H. | 79 | F | O | + | A1, 11, B5, 8 | 32 | 10 | |
| 58 E.N. | 72 | F | O | - | A1, 3, B7, 8 | 32 | | |

Table 2(iii). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|--------------|---------------------------------|--|--------------------------------------|
| 59 O.B. | 73 | F | A | + | A1,2,B8,14 | 32 | | |
| 60 H.W. | 69 | F | B | + | A28,B14,15 | 64 | 3 | |
| 61 J.P. | 78 | F | O | - | A2,9,B7,27 | | 7 | |
| 62 F.W. | 69 | M | A | + | A1,2,B15 | 16 | 4 | |
| 63 H.S. | 66 | M | B | - | A2,B40 | < 16 | 5 | |
| 64 W.C. | 69 | M | A | - | A11,B12,27 | 32 | 6 | AD |
| 65 L.R. | 72 | M | A | - | A1,11,B8,15 | 128 | 3 | |
| 66 H.S. | 72 | M | O | + | A1,B8,13 | 16 | 6 | |
| 67 H.M. | 72 | M | O | + | A1,10,B8 | 16 | | |
| 68 J.P. | 71 | M | O | + | A2,10,B18,40 | 16 | 7 | |
| 69 M.H. | 82 | F | A | - | A1,9,B12,15 | 64 | | |

Table 2(iv). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|--------------|---------------------------------|--|--------------------------------------|
| 70 L.B. | 82 | F | O | - | A2,10,B12 | 64 | | |
| 71 M.G. | 75 | F | O | - | A2,3,B7,17 | 32 | 7 | |
| 72 L.B. | 80 | F | B | + | A2,3,B7,27 | 16 | 6 | |
| 73 S.L. | 73 | F | O | + | A1,2,B7,35 | 32 | 8 | |
| 74 A.T. | 76 | F | O | - | A1,10,B8 | < 16 | | |
| 75 M.M. | 70 | F | A | + | A2,11,B12,15 | 64 | | |
| 76 I.H. | 73 | F | O | + | A2,B12 | 16 | 12 | |
| 77 D.S. | 75 | F | O | - | A1,2,B15,40 | 128 | 5 | AD |
| 78 I.B. | 73 | F | A | + | A3,28,B7,12 | 32 | | |
| 79 T.T. | 79 | M | O | + | A3,11,B7,12 | 128 | | |
| 80 L.H. | 89 | F | AB | + | A2,3,B15 | 256 | 3 | |

Table 2(v). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|-------------|---------------------------------|--|--------------------------------------|
| 81 G.W. | 77 | M | A | - | A2,9B7,17 | 32 | 6 | |
| 82 W.S. | 65 | M | O | - | A3,9,B7,12 | 64 | | |
| 83 H.S. | 78 | F | O | + | A3,9,B7,40 | | 4 | |
| 84 B.C. | 83 | M | A | - | A1,B12,15 | 64 | 6 | AD |
| 85 A.B. | 74 | F | A | - | A2,9,B7,12 | 32 | | |
| 86 C.N. | 75 | F | O | - | A2,B5,12 | 64 | 5 | Mixed AD-multi infarct dementia |
| 87 C.L. | 82 | M | O | + | A1,2,B7,15 | 128 | 7 | |
| 88 G.W. | 70 | F | O | + | A2,9,B5,12 | 16 | 3 | |
| 89 E.P. | 67 | F | AB | + | A2,B15,40 | 32 | | |
| 90 E.W. | 67 | F | O | + | A3,28,B7,12 | | | |
| 91 A.W. | 75 | M | B | + | A1,2,B8 | 64 | 4 | |

Table 2(vi). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|-------------|---------------------------------|--|--------------------------------------|
| 92 M.L. | 74 | F | O | + | A2,B12,15 | 128 | | |
| 93 E.P. | 74 | F | O | + | A2,B7,12 | 32 | | |
| 94 E.C. | 72 | F | B | - | A2,3,B15,27 | 64 | | |
| 95 G.S. | 67 | F | O | + | A2,9,B8,18 | 128 | 9 | |
| 96 A.P. | 70 | F | A | + | A1,9,B8,27 | | 6 | AD |
| 97 E.S. | 73 | F | A | + | A2,3,B12,15 | < 16 | 8 | AD |
| 98 R.W. | 73 | M | O | + | A2,3,B15 | 64 | 5 | AD |
| 99 I.G. | 67 | F | O | - | A2,B12,14 | 32 | 5 | |
| 100 R.K. | 66 | M | A | + | A3,9,B7,40 | 16 | 4 | |
| 101 I.M. | 69 | F | O | + | A3,9,B15,35 | 32 | | |
| 102 F.W. | 71 | M | A | + | A2,B7,12 | 32 | 8 | |

Table 2(vii). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|--------------|---------------------------------|--|--------------------------------------|
| 103 S.C. | 70 | F | O | - | A9,11,B7,12 | 32 | 4 | |
| 104 L.H. | 72 | M | AB | - | A1,2,B8,15 | 64 | 3 | |
| 105 D.B. | 75 | F | A | + | A2,9,B7,8 | < 16 | | |
| 106 A.C. | 70 | F | O | + | A2,11,B15,40 | 32 | 7 | |
| 107 G.F. | 68 | F | A | + | A2,9,B7,12 | 32 | 3 | |
| 108 G.B. | 69 | M | O | + | A2,B12,40 | 32 | | |
| 109 A.L. | 77 | M | O | + | A2,11,B12,40 | 64 | | |
| 110 I.O. | 68 | M | B | + | A1,28,B5 | 16 | 4 | |
| 111 N.B. | 69 | F | B | + | A1,11,B8,15 | 32 | | |
| 112 V.M. | 67 | M | O | + | A10,11,B35 | 32 | 6 | |
| 113 F.L. | 76 | M | O | + | A1,3,B12,35 | 16 | | |

Table 2(viii). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titre (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------|--------------------|-----|-----------------|----------------|-------------|---------------------------------|--|--------------------------------------|
| 114 V.B. | 71 | M | O | + | A3,B7,8 | 32 | 5 | |
| 115 H.T. | 74 | M | O | - | A3,11,B7,40 | 64 | | |
| 116 A.M. | 69 | M | A | - | A1,B8,12 | 16 | 3 | |
| 117 F.C. | 89 | M | AB | + | A2,B5,15 | 128 | 3 | |
| 118 G.O. | 81 | M | O | - | A1,2,B40 | 64 | | |
| 119 W.H. | 83 | M | A | + | A2,3,B17,35 | 128 | 3 | |
| 120 J.W. | 75 | M | A | + | A2,11,B7,8 | 32 | 4 | |
| 121 F.L. | 80 | M | A | + | A1,3B8,22 | 64 | | |
| 122 D.C. | 70 | M | A | + | A9,10,B12 | < 16 | 6 | |
| 123 E.F. | 72 | M | A | - | A2,3,B12,15 | < 16 | 6 | |
| 124 M.M. | 68 | F | O | + | A3,10,B35 | 128 | | |

Table 2(ix). Characteristics of the senile patients

| Initials of patients | Age at onset of AD | Sex | ABO blood group | Rh blood group | HLA type | CMV antibody titres (reciprocal) | Duration of disease (years) in the dead patients | Histological diagnosis at postmortem |
|----------------------------|--------------------------|-----|-----------------------|----------------------|-------------|---|---|--|
| 125 C.W. | | M | O | + | A2, B14, 40 | | | multi-infarct dementia |
| 126 D.H. | 15 | F | | | A9, B15, 17 | 32 | 1 | Creutzfeldt- Jakob disease |

TABLE 3Characteristics of the patients

| | <u>PDAT group</u> | <u>SDAT group</u> |
|---------------------------|-------------------|-------------------|
| Number of patients | 37 | 87 |
| Male : Female | 13 : 24 | 37 : 50 |
| Mean age (years) at onset | 57.3 | 73.5 |
| Range (years) | 45 - 64 | 65 - 89 |
| SD | 5.7 | 5.1 |

TABLE 4Age characteristics of the PDAT patients

| | <u>Males</u> | <u>Females</u> | <u>t value</u> | <u>Significance</u> |
|---------------------------------|--------------|----------------|----------------|---------------------|
| Number | 13 | 24 | | |
| Mean age (years) at onset | 56.62 | 57.63 | 0.510 | $p > 0.5$ |
| Range (years) | 45 - 63 | 49 - 64 | | |
| SD | 6.4 | 5.4 | | |

TABLE 5Age characteristics of the SDAT patients

| | <u>Males</u> | <u>Females</u> | <u>t value</u> | <u>Significance</u> |
|---------------------------------|--------------|----------------|----------------|---------------------|
| Number | 37 | 50 | | |
| Mean age (years) at onset | 74 | 73.06 | 0.855 | 0.5 > P > 0.1 |
| Range (years) | 65-89 | 67-89 | | |
| SD | 5.54 | 4.7 | | |

TABLE 6CMV study - characteristics of the patients and "controls"

| | <u>Patients</u> | <u>Controls*</u> | <u>t value</u> | <u>Significance</u> |
|--|-----------------|------------------|----------------|---------------------|
| Number | 113 | 39 | | |
| Male : Female | 47 : 66 | 23 : 16 | | |
| Mean age (years) at time of study | 73.16 | 70.1 | 1.720 | 0.1>P> 0.05 |
| Range | 48-89 | 54-94 | | |
| SD | 8.959 | 9.425 | | |

* Mean length of hospital admission (years) 23.3 ± 13.89

TABLE 7ABO blood groups in the patients and controls

| | No tested | ABO % | | |
|-----------------------|--------------|----------|-------------|----------|
| | | <u>A</u> | <u>AB+B</u> | <u>O</u> |
| PDAT group | 37 | 48.65 | 5.41 | 45.95 |
| SDAT group | 87 | 37.93 | 13.80 | 48.28 |
| PDAT + SDAT | 124 | 41.13 | 11.29 | 47.58 |
| Controls (493) | 20,719 | 41.65 | 11.41 | 46.94 |
| Leeds BTS Controls | 196 | 38.27 | 13.27 | 48.47 |

TABLE 8Rh blood groups in the patients and controls

| | No tested | Rh % | |
|---------------------------|--------------|-----------------|-----------------|
| | | <u>Rh (D) +</u> | <u>Rh (D) -</u> |
| PDAT group | 37 | 81.08 | 18.92 |
| SDAT group | 87 | 71.26 | 28.74 |
| PDAT + SDAT | 124 | 74.19 | 25.81 |
| Controls ⁽⁴⁹⁴⁾ | 20,719 | 81.8 | 18.2 |
| Leeds BTS Controls | 196 | 83.16 | 16.84 |

TABLE 9(i)HLA ANTIGEN FREQUENCIES IN THE PDAT PATIENTS

| <u>HLA-A</u> | <u>% Controls</u> (n = 458) | <u>% PDAT</u> (n = 37) | <u>% Males</u> (n = 13) | <u>% Females</u> (n = 24) |
|--------------|--------------------------------|---------------------------|----------------------------|------------------------------|
| 1 | 37.11 | 48.65 | 23.08 | 62.50 |
| 2 | 49.12 | 62.16 | 61.54 | 62.50 |
| 3 | 25.11 | 16.22 | 15.39 | 16.67 |
| 9 | 17.90 | 10.81 | 15.39 | 8.33 |
| 10 | 6.55 | 2.70 | 7.69 | - |
| 11 | 12.88 | 8.11 | 23.08 | - |
| 28 | 6.55 | 2.70 | 7.69 | - |

TABLE 9(ii)HLA ANTIGEN FREQUENCIES IN THE PDAT PATIENTS

| <u>HLA-B</u> | <u>% Controls</u> (n = 458) | <u>% PDAT</u> (n = 37) | <u>% Males</u> (n = 13) | <u>% Females</u> (n = 24) |
|--------------|--------------------------------|---------------------------|----------------------------|------------------------------|
| 5 | 9.39 | 10.81 | 15.39 | 8.33 |
| 7 | 29.04 | 24.32 | 38.46 | 16.67 |
| 8 | 25.20 | 13.51 | 7.69 | 16.67 |
| 12 | 30.78 | 29.73 | 30.77 | 29.17 |
| 13 | 3.71 | 5.41 | 7.69 | 4.17 |
| 14 | 8.29 | 10.81 | 15.39 | 8.33 |
| 17 | 9.17 | 5.41 | 7.69 | 4.17 |
| 27 | 7.02 | 8.11 | 7.69 | 8.33 |
| 35 | 12.88 | 18.92 | 15.39 | 20.83 |
| 40 | 9.62 | 13.51 | 7.69 | 16.67 |
| 15 | 13.75 | 32.43* | 23.08 | 37.50 |
| 18 | 8.30 | - | - | - |
| 22 | 2.62 | - | - | - |

* $X^2 = 9.224$, $P = 0.0024$

TABLE 10(i)HLA ANTIGEN FREQUENCIES IN THE SDAT PATIENTS

| <u>HLA-A</u> | <u>% Controls</u> (n = 458) | <u>% SDAT</u> (n = 87) | <u>% Males</u> (n = 37) | <u>% Females</u> (n = 50) |
|--------------|--------------------------------|---------------------------|----------------------------|------------------------------|
| 1 | 37.11 | 29.89 | 37.84 | 24 |
| 2 | 49.12 | 57.47 | 51.35 | 62 |
| 3 | 25.11 | 32.18 | 32.43 | 32 |
| 9 | 17.90 | 25.29 | 16.22 | 32 |
| 10 | 6.55 | 11.49 | 13.51 | 10 |
| 11 | 12.88 | 14.94 | 18.92 | 12 |
| 28 | 6.55 | 6.90 | 2.70 | 10 |

TABLE 10(ii)HLA ANTIGEN FREQUENCIES IN THE SDAT PATIENTS

| <u>HLA-B</u> | <u>% Controls</u> (n = 458) | <u>% SDAT</u> (n = 87) | <u>% Males</u> (n = 37) | <u>% Females</u> (n = 50) |
|--------------|--------------------------------|---------------------------|----------------------------|------------------------------|
| 5 | 9.39 | 6.90 | 5.41 | 8 |
| 7 | 29.04 | 28.74 | 29.73 | 28 |
| 8 | 25.20 | 22.99 | 24.32 | 22 |
| 12 | 30.78 | 36.78 | 32.43 | 40 |
| 13 | 3.71 | 1.15 | 2.70 | - |
| 14 | 8.29 | 4.60 | - | 8 |
| 17 | 9.17 | 5.75 | 8.10 | 4 |
| 27 | 7.02 | 9.20 | 5.41 | 12 |
| 35 | 12.88 | 14.94 | 10.81 | 18 |
| 40 | 9.62 | 13.79 | 18.92 | 10 |
| 15 | 13.75 | 26.44* | 29.73 | 24 |
| 18 | 8.30 | 3.45 | 2.70 | 4 |
| 22 | 2.62 | 1.15 | 2.70 | - |

* $\chi^2 = 8.837$, $p = 0.003$

TABLE 11(i)

HLA ANTIGEN FREQUENCIES IN THE COMBINED
(PDAT + SDAT) GROUP OF PATIENTS

| <u>HLA-A</u> | <u>% Controls</u> (n = 458) | <u>% Combined</u> (n = 124) | <u>% Males</u> (n = 50) | <u>% Females</u> (n = 74) |
|--------------|--------------------------------|--------------------------------|----------------------------|------------------------------|
| 1 | 37.11 | 35.48 | 34 | 36.49 |
| 2 | 49.12 | 58.90 | 54 | 62.16 |
| 3 | 25.11 | 27.42 | 28 | 27.03 |
| 9 | 17.90 | 20.97 | 16 | 24.32 |
| 10 | 6.55 | 8.87 | 12 | 6.76 |
| 11 | 12.88 | 12.90 | 20 | 8.10 |
| 28 | 6.55 | 5.65 | 4 | 6.76 |

TABLE 11(ii)

HLA ANTIGEN FREQUENCIES IN THE COMBINED
(PDAT + SDAT) GROUP OF PATIENTS

| <u>HLA-B</u> | <u>% Controls</u> (n = 458) | <u>% Combined</u> (n = 124) | <u>% Males</u> (n = 50) | <u>% Females</u> (n = 74) |
|--------------|--------------------------------|--------------------------------|----------------------------|------------------------------|
| 5 | 9.39 | 8.07 | 8 | 8.10 |
| 7 | 29.04 | 27.42 | 32 | 24.32 |
| 8 | 25.20 | 20.16 | 20 | 20.27 |
| 12 | 30.78 | 34.68 | 32 | 36.49 |
| 13 | 3.71 | 2.42 | 4 | 1.35 |
| 14 | 8.29 | 6.45 | 4 | 8.10 |
| 17 | 9.17 | 5.65 | 8 | 4.05 |
| 27 | 7.02 | 8.87 | 6 | 10.81 |
| 35 | 12.88 | 16.13 | 12 | 18.92 |
| 40 | 9.62 | 13.71 | 16 | 12.16 |
| 15 | 13.75 | 28.20* | 28 | 28.38 |
| 18 | 8.30 | 2.42 | 2 | 2.70 |
| 22 | 2.62 | 0.81 | 2 | - |

* $\chi^2 = 14.619$, $P < 0.001$

Figure 2 - Frequency of HLA-B15 in Relation to Age at Onset

of Alzheimer's disease

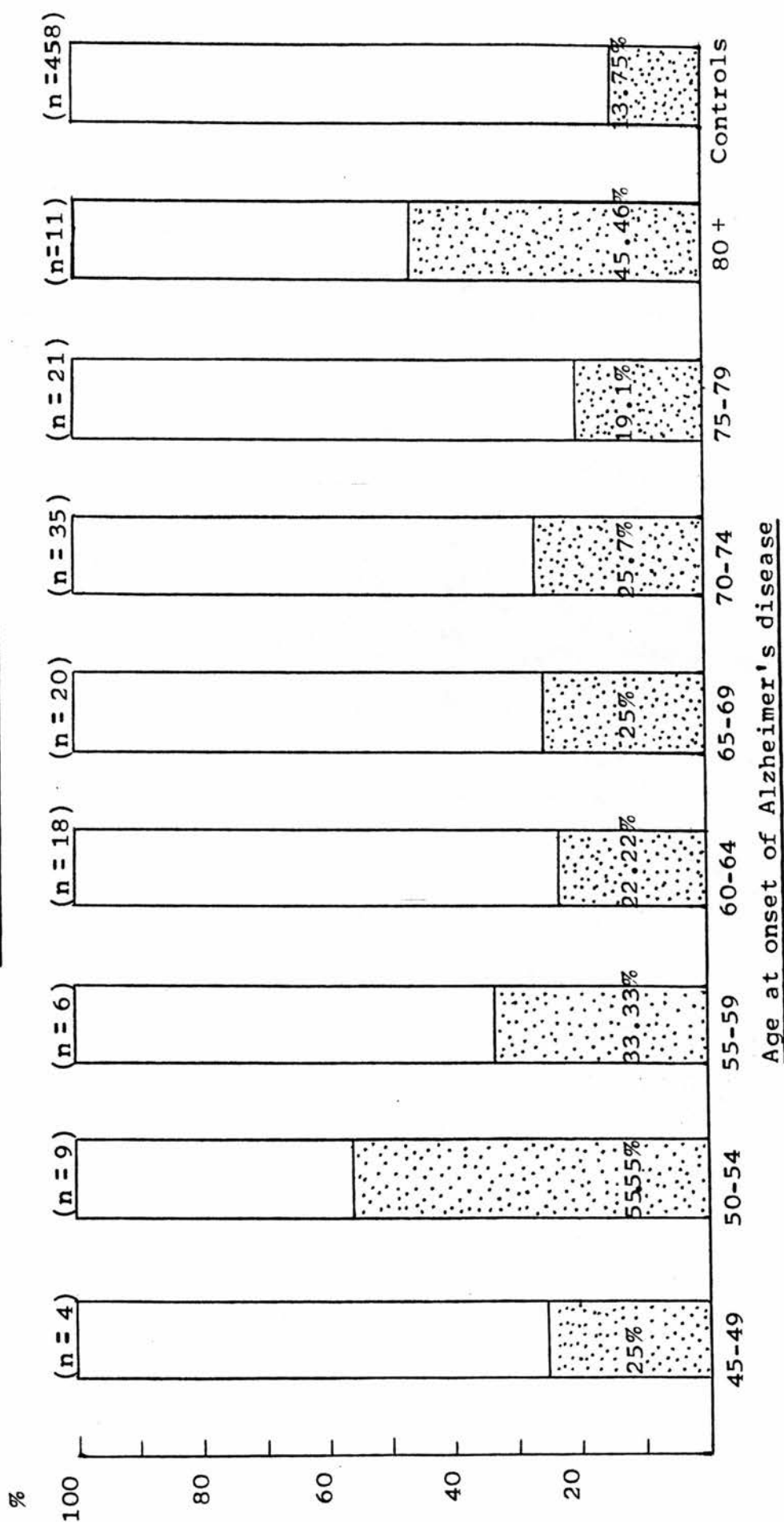


TABLE 12

Age characteristics of the HLA-B15 positive
and HLA-B15 negative PDAT patients

| | <u>HLA-B15⁺</u> | <u>HLA-B15⁻</u> | <u>t value</u> | <u>Significance</u> |
|---------------------------------|----------------------------|----------------------------|----------------|---------------------|
| Number | 12 | 25 | | |
| Mean age (years) at onset | 56.08 | 57.84 | 0.878 | 0.5 > P > 0.1 |
| Range (years) | 49-64 | 45-64 | | |
| SD | 5.5 | 5.8 | | |

TABLE 13

Age characteristics of the HLA-B15 positive
and HLA-B15 negative SDAT patients

| | <u>HLA-B15+</u> | <u>HLA-B15-</u> | <u>t value</u> | <u>Significance</u> |
|---------------------------------|-----------------|-----------------|----------------|---------------------|
| Number | 23 | 64 | | |
| Mean age (years) at onset | 74.96 | 72.92 | 1.674 | 0.1 > P > 0.05 |
| Range (years) | 67 - 89 | 65 - 83 | | |
| SD | 6.321 | 4.467 | | |

TABLE 14

Length of history of AD in deceased
HLA-B15 positive and HLA-B15
negative PDAT patients

| | <u>HLA-B15+</u> | <u>HLA-B15-</u> | <u>t value</u> | <u>Significance</u> |
|-----------------------------|-----------------|-----------------|----------------|---------------------|
| Number | 5 | 9 | | |
| Mean duration (years) | 10 | 8.1 | 0.8539 | $0.5 > P > 0.1$ |
| Range (years) | 4 - 15 | 4 - 15 | | |
| SD | 4 | 3.983 | | |

TABLE 15Length of history of AD in deceasedHLA-B15 positive and HLA-B15negative SDAT patients

| | <u>HLA-B15+</u> | <u>HLA-B15-</u> | <u>t value</u> | <u>Significance</u> |
|-----------------------------|-----------------|-----------------|----------------|---------------------|
| Number | 16 | 34 | | |
| Mean duration (years) | 5 | 5.8 | 1.2316 | 0.5 > P > 0.1 |
| Range (years) | 3 - 9 | 3 - 12 | | |
| SD | 1.967 | 2.104 | | |

Table 16(i). Family history of AD in 10 HLA-B15 positive and 10 HLA-B15 negative patients matched for sex and age at onset of disease

| Number of patient | Initials of patient | HLA type | Sex | Age at onset | Family history | Number of first degree relatives over 44 years of age | Affected relatives |
|-------------------|---------------------|----------|-----|--------------|----------------|---|---|
| (6) | J.N. | B15 + | M | 51 | - | 7 | - |
| (1) | L.M. | B15 - | M | 52 | +? | 7 | Father ? (SDAT) |
| (3) | C.O. | B15 + | F | 64 | + | 1 | Mother (PDAT) |
| (4) | E.S. | B15 - | F | 63 | - | 4 | - |
| (12) | F.S. | B15 + | F | 55 | + | 7 | Father (SDAT) - (confirmed by death certificate.) |
| (8) | N.B. | B15 - | F | 55 | - | 1 | - |
| (22) | O.S. | B15 + | F | 49 | - | 3 | - |
| (10) | M.S. | B15 - | F | 49 | - | 6 | - |
| (69) | M.H. | B15 + | F | 82 | + | 7 | Sister (SDAT)* - (confirmed by personal examination.) |
| (41) | D.S. | B15 - | F | 81 | - | 7 | - |
| (53) | W.W. | B15 + | M | 78 | - | 4 | - |
| (79) | T.T. | B15 - | M | 79 | - | 10 | - |

Table 16(ii). Family history of AD in 10 HLA-B15 positive and 10 HLA-B15 negative patients matched for sex and age at onset of disease

| Number of patient | Initials of patient | HLA type | Sex | Age at onset | Family history | Number of first degree relatives over 44 years of age | Affected relatives |
|-------------------|---------------------|--------------|--------|--------------|----------------|---|--------------------|
| (77 (86 | D.S. C.N. | B15+ B15- | F F | 75 75 | - - | 2 5 | - - |
| (89 (90 | E.P. E.W. | B15+ B15- | F F | 67 67 | - + | 8 5 | - Father (PDAT) |
| (101 (42 | I.M. E.S. | B15+ B15- | F F | 69 69 | + - | 6 6 | Mother (SDAT) - |
| (106 (47 | A.C. M.T. | B15+ B15- | F F | 70 70 | - - | 5 9 | - - |

* HLA type A1,9,B12,15.

Table 17(i). Family history of AD in a further 14 HLA-B15
positive patients

| Number of patient | Initials of patient | Sex | Age at onset | Family history | Number of first degree relatives over 44 years of age | Affected relatives |
|-------------------------|---------------------------|-----|--------------------|-------------------|--|---|
| 16 | I.S. | F | 54 | - | 3 | - |
| 37 | M.R. | F | 50 | - | 1 | - |
| 45 | B.L. | M | 78 | - | 3 | - |
| 50 | H.H. | M | 77 | - | 4 | - (Mother died of ? leukaemia) |
| 62 | F.W. | M | 69 | + | 5 | Mother (PDAT); Brother (PDAT)** - (confirmed by personal examination); Brother (SDAT) - (confirmed by hospital case notes) |
| 65 | L.R. | M | 72 | + | 9 | Mother (PDAT) - (confirmed by hospital case notes) |
| 75 | M.M. | F | 70 | - | 3 | - |
| 80 | L.H. | F | 89 | - | 2 | - |

Table 17(ii). Family history of AD in a further 14 HLA-B15 positive patients

| Number of patient | Initials of patient | Sex | Age at onset | Family history | Number of first degree relatives over 44 years of age | Affected relatives |
|-------------------|---------------------|-----|--------------|----------------|---|---|
| 87 | C.L. | M | 82 | + | 8 | Two brothers (SDAT) |
| 92 | M.L. | F | 74 | - | 5 | - |
| 94 | E.C. | F | 72 | + | 8 | Father (SDAT); Sister (PDAT) |
| 97 | E.S. | F | 73 | + | 6 | Father (SDAT) |
| 104 | L.H. | M | 72 | - | 3 | (Father shot himself in his early sixties. Sister died of ? leukaemia). |
| 123 | E.F. | M | 72 | + | 5 | Mother (SDAT); Father (SDAT) |

** HLA type A2,B15,35

TABLE 18

Complement-fixing antibody titres
to CMV in patients and "controls"

| | % of patients with reciprocal serum antibody titres to CMV | | | | | |
|---------------------------------------|---|---------------|---------------|---------------|---------------|--------------|
| | < 16 | 16 | 32 | 64 | 128 | 256 |
| SDAT + PDAT patients* (n = 113) | 14.16 (16) | 19.47 (22) | 30.97 (35) | 23.89 (27) | 10.62 (12) | 0.885 (1) |
| Controls** (n = 39) | 23.08 (9) | 20.51 (8) | 17.95 (7) | 33.33 (13) | 5.13 (2) | |

* Mean age at time of study = 73.16 years \pm 8.959

** Mean age at time of study = 70.1 years \pm 9.425

TABLE 19

Serum geometric mean antibody titres
(log₁₀) to CMV in patients and 'controls'

| | <u>Patients</u> | <u>Controls</u> | <u>t value</u> | <u>Significance</u> |
|-------------------------|-----------------|-----------------|----------------|---------------------|
| Geometric mean titre | 32 | 27.27 | | |
| (log ₁₀) | 1.5051 | 1.4357 | 0.9963 | 0.5 > P > 0.1 |
| | ± 0.3709 | ± 0.3878 | | |

TABLE 20

Complement-fixing antibody titres
to CMV in HLA-B15 positive and
negative patients

| % of patients with reciprocal serum antibody titres to CMV | | | | | | |
|---|--------------|--------------|---------------|---------------|-------------|------------|
| | < 16 | 16 | 32 | 64 | 128 | 256 |
| HLA-B15+ patients* (n = 33) | 12.1 (4) | 6.1 (2) | 24.2 (8) | 36.4 (12) | 18.2 (6) | 3.0 (1) |
| HLA-B15- patients** (n = 80) | 15.0 (12) | 25.0 (20) | 33.75 (27) | 18.75 (15) | 7.5 (6) | |

* Mean age of B15 positives = 73.1 years \pm 9.992

**Mean age of B15 negatives = 73.2 years \pm 8.566

TABLE 21

Serum geometric mean antibody
titres (\log_{10}) to CMV in B15 positive
and negative patients

| | <u>HLA-B15+</u> | <u>HLA-B15-</u> | <u>t value</u> | <u>Significance</u> |
|-------------------------|-----------------|-----------------|----------------|---------------------|
| Geometric mean titre | 45.73 | 27.62 | | |
| (\log_{10}) | 1.6602 | 1.4412 | 2.952 | 0.01 > P > 0.001 |
| | \pm 0.392 | \pm 0.3444 | | |

11) Creutzfeldt-Jakob disease in an HLA-B15 positive
15 year old girl:

Case history:

The patient D.H. (patient number 126) a female of West Indian parentage, developed a rapidly progressive illness at the age of 15, characterised by dementia, myoclonic jerks, spasticity of the limbs, and muscle wasting, and died 1 year later.

Tests revealed that she was HLA-B15 positive, and that she had a CMV antibody titre of 1/32, and a polyomavirus BK titre of $\geq 1/20,480$.

Post-mortem results:

Histological examination of the brain was made by Dr. Harriman, Consultant Neuropathologist at Leeds General Infirmary, who reported:

"Cerebral cortex: There is widespread spongiform change, variable in intensity but severe in the frontal, parietal and occipital regions. The "ground substance" (i.e. neuronal and glial cell processes) of the grey matter is pitted by numerous spaces of variable size, rarely much larger than a neuron. Some contain shrunken neurons or glial cells. There is considerable loss of neurons from all laminae, but astrocytes proliferate to give the cortical ribbon an appearance of excess cellularity. A small proportion of the astrocytes are in the swollen form. These changes lead to disorganization of the laminar pattern. The temporal and insular

cortices are least affected, and the cornu ammonis escapes completely. Silver stains of the frontal cortex confirm the gliosis, and show no evidence of senile plaque formation or neurofibrillary tangles.

White matter: This is mostly normal. The exception lies in the parietal white matter, where there is diffuse myelin pallor, occasional vacuolation of axons and astrocytosis.

Basal ganglia: Spongiform change with neuronal loss, and less gliosis than in the cortex, affects the head of the caudate nucleus and the putamen. Both large and small neurons are affected, predominantly the latter. The change is sharply limited to these structures, no pathological features being found in the globus pallidus and substantia nigra.

Mid and brain stem: The pons and medulla are normal. In the cerebellum, the dentate nucleus is not affected. The folia show separation of the molecular zone from the granular layer, and the granular cells are rarefied. This is attributed to artefact.

Comment: Thus the histopathological features are those of subacute spongiform encephalopathy. It corresponds with the morphological characteristics of Creutzfeldt-Jakob disease despite the fact that the age of the patient is at the lowermost limit of the age incidence of that condition."

No virus particles were seen on electron microscopy and no viruses were isolated after 5 months incubation.

12) PDAT in one of monozygotic twin sisters:

Case History:

The patient M.S. (patient number 10) was admitted to High Royds hospital on the 15th January 1973 at the age of 57 suffering from dementia.

According to her relatives, her illness started insidiously about 1964, when she was 49 years of age. At that time she began to forget the names of friends, and started to jot down information on pieces of paper to remind her to do her household duties, or remember where she had placed various objects. Later, she started to buy unnecessary goods from shops, and began to neglect her housework. As her memory loss became worse, she had difficulty in following conversations, and became easily frustrated and tearful when she got things wrong.

She was admitted to a general hospital psychiatric unit for investigation of her memory loss in August 1968 when she was 53. On examination, she was noted to be a neatly dressed grey haired woman, who smiled blandly and at times was euphoric. She gesticulated a lot, and tended to fiddle with her ring when she had difficulty in answering questions. She stuttered considerably and

interjected a lot of "you know's" to cover up her difficulties in expression or answering questions. She was very vague, and it was impossible to get a coherent history from her.

Her concentration was poor, and she showed severe impairment of long-term, and to a lesser extent, short-term memory. Her general information was poor, and she was disorientated in time, place, and person. She showed some degree of sensory, nominal and expressive dysphasia, and constructional apraxia, and she had gross dyscalculia. There was also evidence of perseveration and confabulation.

Physical examination showed a plump well-nourished normotensive woman with moderate arcus senilis. No obvious abnormalities were found, although it was noted on neurological examination, that her reflexes were slightly brisker on the left.

A full blood count, ESR, urea and electrolytes, liver function tests, blood glucose, serum cholesterol, thyroid function tests, serum folate and vitamin B₁₂ were normal. A WR and Kahn were negative, and skull and chest X-rays, ECG, and CSF examination including a Lange, were normal. An AEG, left carotid angiogram and radioactive isotope brain scan were also normal. A series of EEG records were abnormal, being dominated by activity of theta and delta frequency symmetrically distributed, and in one EEG, subclinical seizure activity was observed.

She was discharged from hospital on 18th October 1968 and her condition continued to deteriorate over the next four years. Her memory deteriorated, she lost her capacity to wash or dress herself, and she had to be fed. She rarely spoke and usually sat all day staring vacantly into the fire or through the window, although would sometimes join in the hymn singing on the radio.

Her husband and twin sister devotedly attended to all her needs, but eventually the task proved too much, and she was admitted to High Royds hospital in January 1973.

Although occasionally agitated and restless at times initially, she later became mute, unresponsive and doubly incontinent. There was a generalized increase in muscle tone, reflexes were brisk and symmetrical, and plantar responses were flexor. Later, twitching movements of the limbs were observed. For about a year before death, she had difficulty in swallowing, and her weight steadily declined. She finally became bedridden and required total nursing care. She died on Boxing Day 1979, of bronchopneumonia, about 15 years after the onset of dementia.

Family History of the Twins:

Their parents were unrelated. Both were healthy, although their mother suffered from insulin-dependent diabetes in later life. Their father died at the age

of 65 from pneumonia, and their mother died aged 75 from heart and renal failure.

Their older sister, and younger brother and sister are alive and well, and there is no family history of dementia.

Personal History of the Twins:

Minnie, the patient, and her twin Annie, were born full-term, after an uneventful delivery. Each weighed about 6 lbs., and the patient was the second born.

Their childhood was happy. Both were good scholars, and always achieved similar marks in examinations. They were identical in appearance, and had similar mannerisms. However the patient was the more active of the two, and in her early adulthood, went rock-climbing and pot-holing. Minnie was also more serious and conscientious than her sister. On leaving school, they worked at the same mill as weavers although Minnie was always the better and faster worker. They were constant companions until Annie married at 21, and moved away. Minnie married at 22, and never moved out of the area where she was born.

Although parted geographically, the twins remained very close and visited each other regularly, and went on holiday together.

Minnie had a daughter now aged 38 who remains well. Annie also has a daughter aged 40, who has had treatment

for Hodgkin's disease.

The patient had no serious physical or psychiatric illnesses prior to the onset of AD, although in later adulthood became easily upset by stress. Annie has remained well, and showed no evidence of dementia 18 months after the death of her twin sister.

Confirmation of monozygosity:

The twins were identical in physical appearance (Figures 3 (a), (b) and (c)), and were frequently mistaken for each other.

The results of blood group studies on the twins shown in Table 22, also strongly suggest monozygosity.



Fig. 3 (a). The twins in infancy.



Fig. 3 (b). The twins in childhood.



Fig. 3 (c). The twins in adulthood.

TABLE 22Results of blood group studies of the twins

| <u>Blood group</u> | <u>Initials of twins</u> | |
|----------------------|--------------------------|-----------------|
| <u>system</u> | <u>M.S.</u> | <u>A.H.</u> |
| ABO | O | O |
| Rh(D) | + | + |
| Phenotype | ccDEe | ccDEe |
| Direct Anti-Globulin | - | - |
| MS | MS _S | MS _S |
| P ₁ | - | - |
| Lu ^a | - | - |
| K | - | - |
| Le ^a | - | - |
| Le ^b | + | + |
| Fy ^a | - | - |
| Jk ^a | + | + |
| Jk ^b | + | + |

The total chance of dizygosity in caucasian twins with the above blood groups is approximately 0.02.

| | | |
|--------------|---------|---------|
| HLA antigens | A1,B17, | A1,B17, |
| | 35 | 35 |

The probability of M.S. and A.H. being dizygotic with the above HLA antigens is 0.25.

Summary of autopsy findings:

" The body was that of an emaciated elderly woman. All the visceral organs were underweight and there was a severe bronchopneumonia.

The brain weighed 850 g and externally showed marked generalized cortical atrophy which was maximal at the frontal poles (Figures 4 (a), (b) and (c)). On coronal sectioning the cerebral ventricles were dilated and the cerebral cortex thinned especially in the occipital region (Figures 5 (a), (b) and (c)). The subcortical structures appeared relatively normal.

Microscopically, senile plaques were found in the cerebral cortex in representative sections stained by haematoxylin and eosin from the temporal, frontal, parietal and occipital regions. Frozen sections of the temporal lobe, stained by Von Braumuhls silver impregnation technique, showed that these plaques were very numerous and many of the cortical neurons showed neurofibrillary tangles (Figure 6).

The appearances are consistent with the clinical diagnosis of dementia of the Alzheimer type. "



Fig. 4 (a). Superior aspect of the brain of M.S., showing generalized cerebral cortical atrophy, with thinning of the gyri, and widening of the sulci.

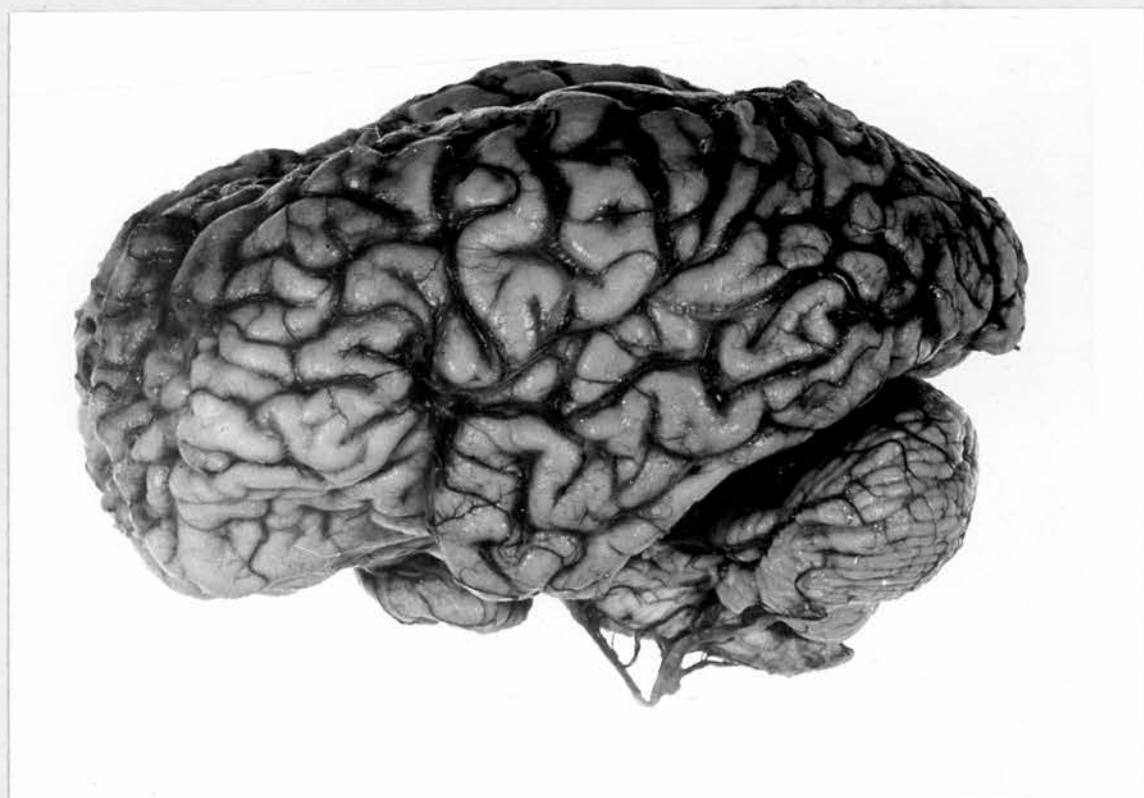


Fig. 4 (b). Lateral aspect of the brain of M.S., showing generalized cerebral cortical atrophy, with thinning of the gyri, and widening of the sulci.

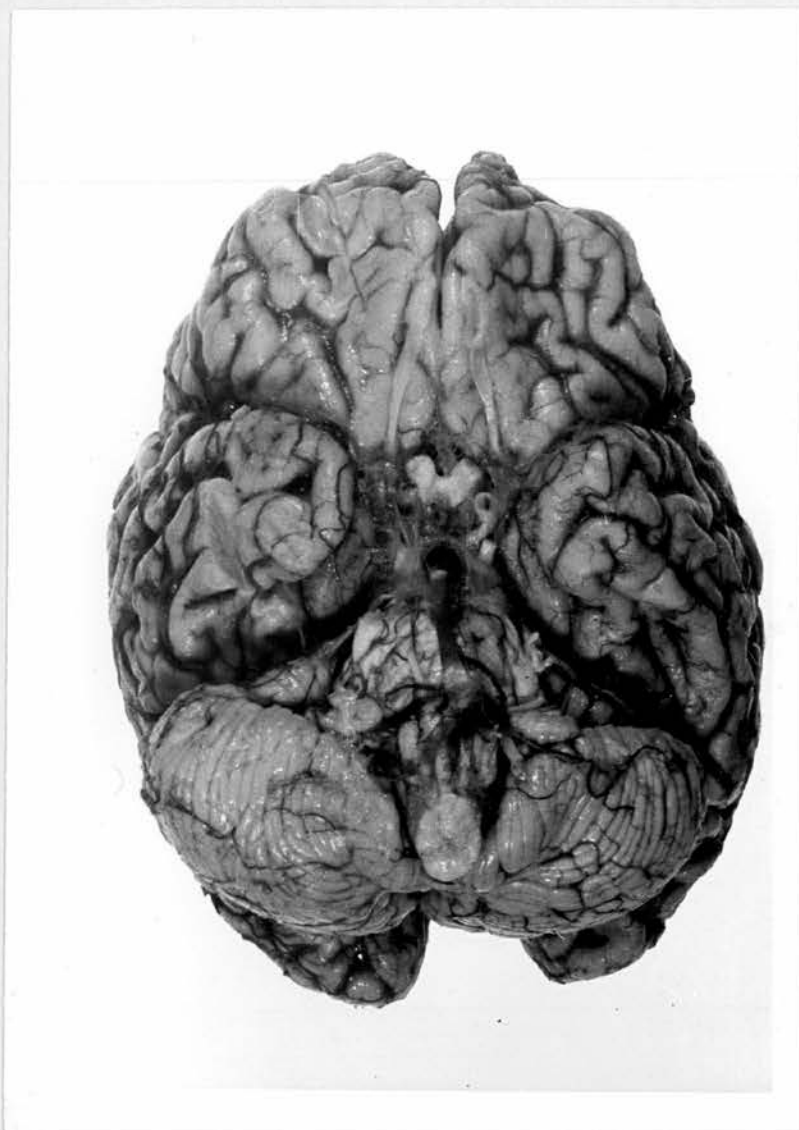


Fig. 4 (c). Inferior aspect of the brain of M.S., showing generalized cerebral cortical atrophy, with thinning of the gyri, and widening of the sulci.

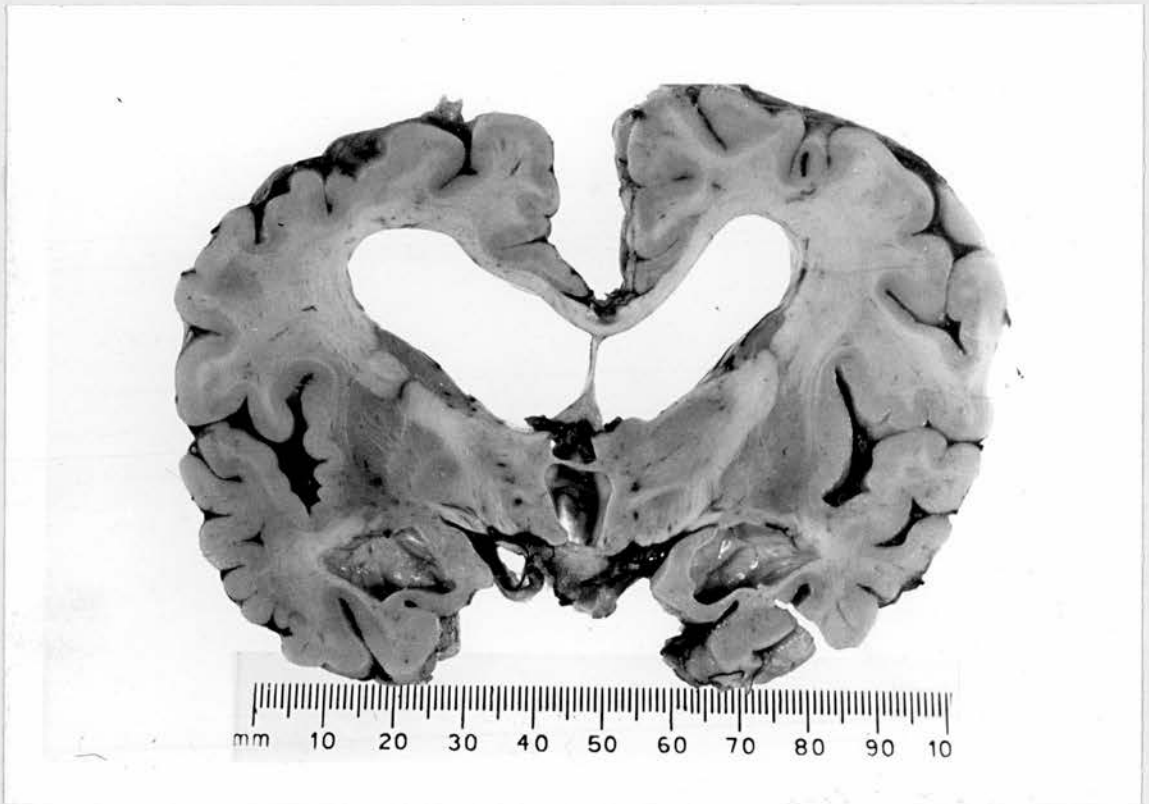


Fig. 5 (a). Coronal section through the fronto-parietal region of the cerebral hemispheres of M.S. showing cortical thinning and ventricular dilatation.

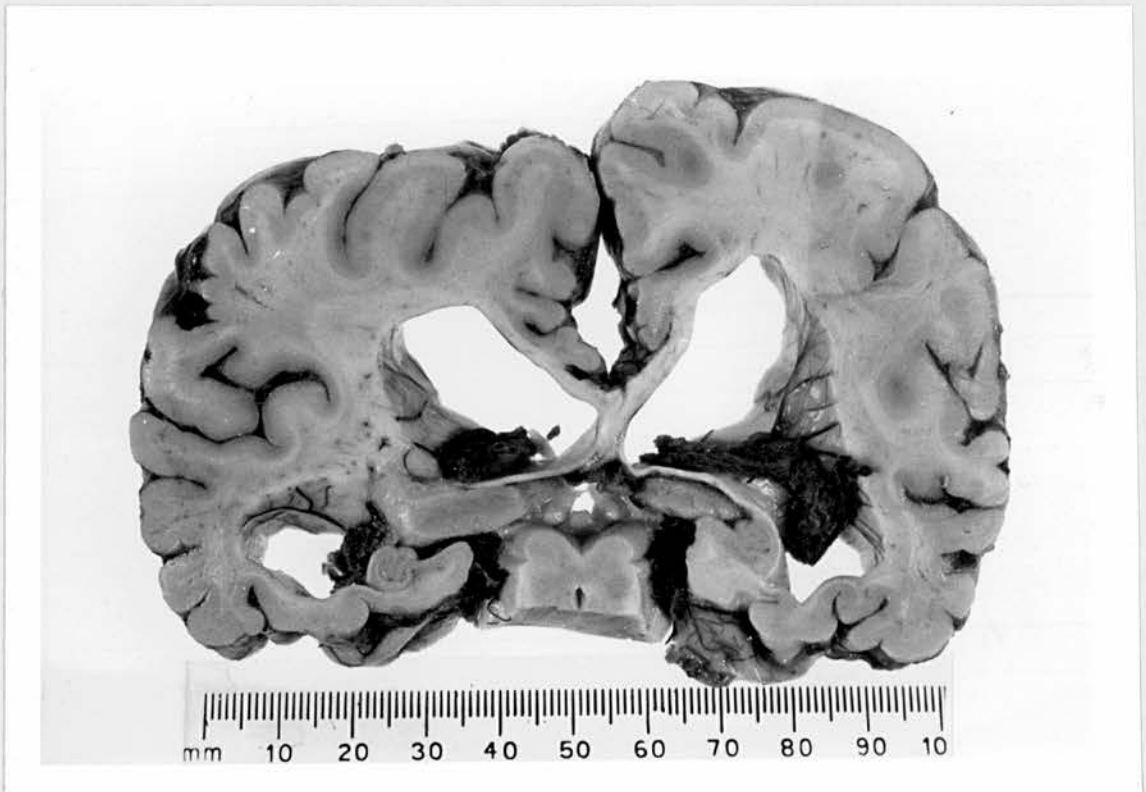


Fig. 5 (b). Coronal section through the parietal region of the cerebral hemispheres of M.S., including Ammon's Horn, showing cortical thinning and ventricular dilatation.

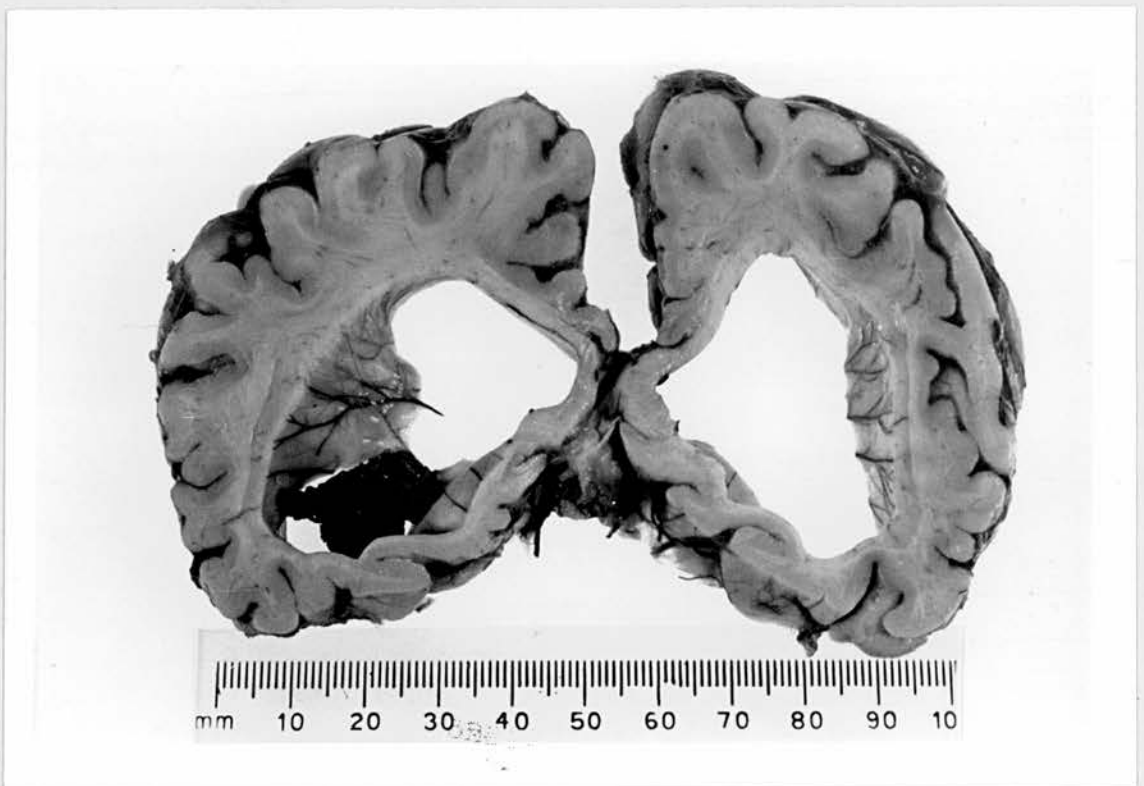


Fig. 5 (c). Coronal section through the occipital region of the cerebral hemispheres of M.S., showing cortical thinning and ventricular dilatation.

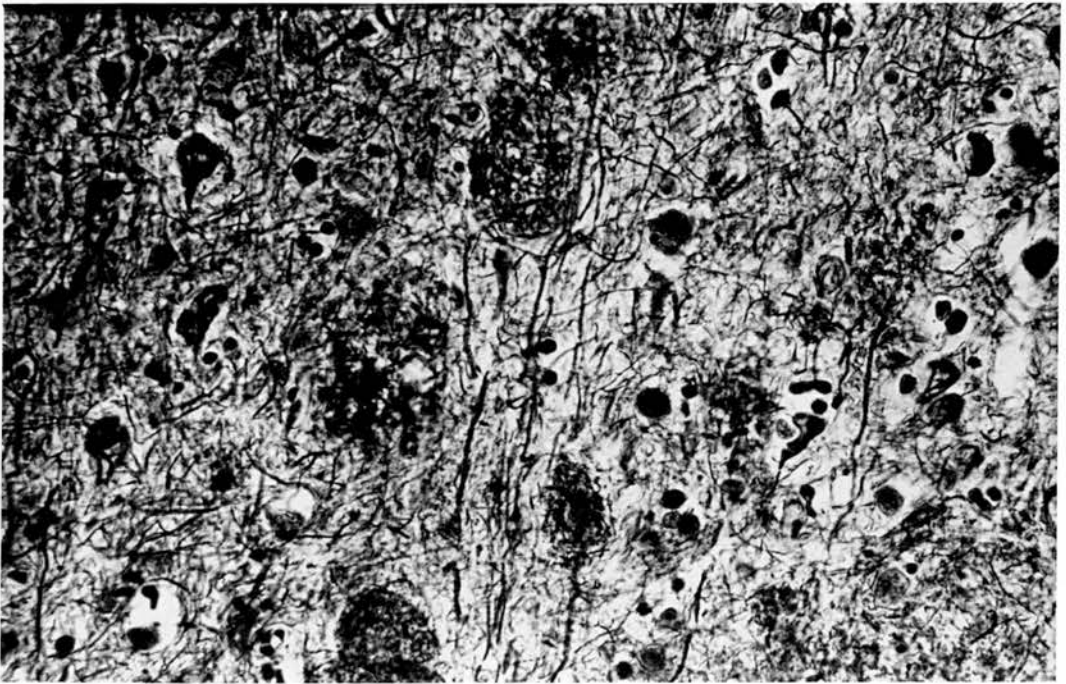


Fig. 6. Section of the temporal lobe of M.S., stained by Von Braumuhl's silver impregnation technique, showing senile plaques and neurofibrillary tangles. X 100.

DISCUSSION

METHODOLOGY

DISCUSSIONMETHODOLOGY

1) THE CLINICAL DIFFERENTIATION OF ALZHEIMER'S
DISEASE FROM OTHER FORMS OF DEMENTIA:

Unfortunately only a clinical diagnosis of AD can be made during life. Cerebral biopsy is now considered to be unethical, and anyway does not always provide an accurate histological diagnosis. It is therefore necessary at this point to consider the evidence in support of the validity of differentiating AD from other forms of dementia on clinical features alone.

About 85% of the cases of dementia occurring in the elderly are due to SDAT, multi-infarct dementia (MID), in which brain damage occurs as the result of multiple small or large infarcts usually from embolization, or a mixture of both diseases (87,495-497). In the other 15% of cases dementia results from other pathological processes, and most of these should have been screened out from this study.

Although frequently overdiagnosed clinically, MID (498) is distinctly less common than SDAT which is responsible for the majority of cases of dementia occurring after the age of 64 (87, 495-497).

Certain clinical features are traditionally held to differentiate between SDAT and MID (499-501):

In SDAT, the onset is usually insidious, and insight is soon lost. Typically there is early deterioration of memory and personality, and mood changes such as irritability and blunting of emotion may occur. The course of the disease is more gradual than MID, and the incidence of hypertension is no greater than expected in the general population of a similar age. Neurological signs usually appear late.

However in MID, the onset is often fairly sudden, but insight, personality and memory are relatively well preserved until the later stages of the disease. Anxiety, depression and emotional incontinence may occur, and hypertension is frequently present. The course of MID is fluctuating and often step-like in association with cerebrovascular accidents and acute episodes of clouding of consciousness. Focal neurological signs typically appear early in the disease, and evidence of generalized arteriosclerosis may be found.

These traditional clinical criteria for distinguishing between SDAT and MID which were used in the present study, have been validated by recent clinico-pathological studies.

Corsellis (495) found broad agreement between the clinical diagnosis of SDAT and the extent of senile plaques and neurofibrillary tangles in the brain, and also observed a similar relationship between MID and the degree of cerebral vascular changes. However changes of both SDAT and MID were found in about 20% of the brains.

The clinical and pathological distinctions between SDAT and MID were also upheld by the Newcastle workers (13,84) in a prospective study of demented patients, although they too found that about 20% of the brains had mixed SDAT-MID changes (87). They showed that the severity of cognitive impairment during life is quantitatively correlated with mean senile plaque counts, and severity of neurofibrillary changes in SDAT patients, and the amount of cerebral softening in MID patients (84). Threshold effects of pathological change were demonstrable in both types of dementia below which the brain could accommodate without obvious intellectual and personality deterioration but beyond which the clinical picture of dementia is likely to occur (84). For SDAT, the threshold value for mean plaque counts was 14 plaques per field, and for MID, 50 mls of cerebral softening (84). Ninety four per cent of the patients with a clinical diagnosis of SDAT were found to have a total volume of cerebral softening less than 50 mls, but 73% of patients with a clinical diagnosis of MID had a total of more than 50 mls of cerebral softening (84).

In an attempt to quantify the MID changes in demented patients, Hachinski et al (502,503) have designed a scale based on the traditional criteria for MID, which rates each patient on an ischaemic score, and correlated these with cerebral blood flow. Cerebral blood flow was found to be normal in SDAT but reduced in MID, and the degree of dementia was inversely related to

the flow. They conclude that a patient's ischaemic score successfully differentiates between SDAT and MID, and that the score differences reflect the greater degree of cerebral ischaemic damage in MID.

PDAT is the most common form of presenile dementia (13), but has to be differentiated from Pick's disease which is much less common. Other forms of presenile dementia such as CJD and Huntington's chorea are rare.

Traditionally PDAT has been differentiated from Pick's disease on specific clinical features. Memory impairment, parietal lobe symptoms such as dysphasia, apraxia, and agnosia, and disturbances in gait and muscle tone are said to occur early in PDAT (10); in Pick's disease, frontal lobe symptoms are described as dominating the early picture with personality deterioration, emotional blunting, loss of drive and insight, disinhibited behaviour, antisocial conduct and incontinence often being present (504). Memory and intellect are usually well preserved in the early stages.

Many of these traditional criteria have been validated by recent clinico-pathological studies. Sim and coworkers (505-507) performed cerebral biopsies on 56 patients with presenile dementia, and found that memory impairment, apraxia, and EEG changes occurred early in PDAT, but late in Pick's disease, whereas personality changes, incontinence, fits, psychotic features, confabulation, and neurological signs occurred early in Pick's but late in PDAT. Todorov et al (508) successfully diagnosed PDAT

clinically in 28 out of 32 presenile patients who underwent post-mortems, using the traditional clinical criteria of a dementia occurring insidiously before the age of 65, with early memory and intellectual deterioration, aphasia, agnosia and apraxia with typical evolution of the disease. Similar clinical criteria were also used in this study for the diagnosis of PDAT.

There is therefore clear evidence that AD can be clinically diagnosed with reasonable success using traditional clinical criteria, but some reservations need to be made. The clinical differentiation of SDAT from MID is straight forward when massive focal symptomatology is present, but is more difficult when these features are less prominent. Also the differentiation of PDAT from Pick's disease becomes less easy with time, as their clinical features tend to merge.

It is perhaps inevitable that some of the patients included in this study did not have AD. However the histological confirmation of the disease in most of the patients who underwent post-mortems would suggest that their numbers are small, and although diluting the results, should not significantly affect their validity.

2) THE PATIENT POPULATION STUDIED

The use of hospital patients in this study should not limit the application of its findings. Kay, Beamish and Roth (17) reported little difference in the severity of dementia of patients in hospitals and institutions and those living in the community, and found that social and family factors play a more important role in determining admission than severity of illness. Inpatients with AD are therefore fairly representative of the general population of patients with this disease, and conclusions drawn from this study should be able to be applied generally.

However it is important to recognize that spurious associations between blood groups and diseases can result from genetic stratification in the population, when the patients belong to a subpopulation in which the blood group and disease may be more frequent than in the control group, although they have no causal connection. Genetic stratification can be ruled out as an explanation if similar blood group and disease associations are found in populations of different ethnic origins.

3) IDENTIFICATION OF SECONDARY CASES

In the family studies, the patient's next of kin had to be relied on to provide up-to-date information on their relatives, which in some cases they were not fully able to do.

Corroboration of secondary cases of dementia with hospital records and death certificates proved impossible in most cases. Most of the secondary cases of dementia had died or were living some distance away, and only 2 could be personally examined. Independent corroboration of the disease was obtained in 6 of the 17 secondary cases of dementia. In 3 cases the disease was corroborated from hospital case notes, in 2 cases from hospital case notes and personal examination, and in a further case from a death certificate.

Although included as an affected relative, there is an element of doubt about the diagnosis of the father of HLA-B15 negative patient number 8. The father's hospital case notes show that he started to dement in his late 60's about a year after undergoing a prefrontal leucotomy, which was performed to alleviate his chronic depressive illness.

Some of the secondary cases of dementia had never in fact been admitted to hospital, and the hospital records of others who had been, were destroyed a few years after their death and were therefore unavailable for scrutiny.

Death certificates were also found to be an

unreliable means of corroborating dementia. Heston and Mastri (102) reported similar difficulties, and found that in 17 out of 30 patients with histological evidence of AD, the causes of death on their death certificates were given as "cerebral vascular disease" or "pneumonia".

The difficulties associated with such studies indicate that conclusions about the frequency of AD in the families of the patients can only be tentative.

4) THE CHOICE OF THE CONTROL GROUPS

The proper choice of a control group is of the greatest importance in blood group and disease association studies, and the controls should be drawn from a population with a similar ethnic background as the patients. New blood donors are frequently used as controls although are not entirely satisfactory. They are self-selected and therefore it is impossible to be sure how representative they are of the general population, particularly as their home addresses and not their places of birth are recorded. Self-selection may have the particular effect of increasing the frequency of Rh negatives among new blood donors, especially women.

The controls used for the blood group studies are considerably younger than my patients, but this age

difference should not invalidate the results. Although Vogel (509) has claimed that people who are blood group O are healthier and live longer than those who are blood group A, other workers (510,511) have found no significant alterations in ABO and Rh blood group frequencies with increasing age. Also Bender et al (512), and Hansen, Sparck and Larsen (513), reported that HLA frequencies did not alter significantly with increasing age either, although Macurova et al (514) found that HLAw10 (now B40) was increased in people over 80 years of age (28.91%) compared with adults (9.71%) and youths (10%). Yarnell and colleagues (511) also found good general agreement in HLA antigen frequencies between a random sample of elderly people and a control group, although they too reported an excess frequency of HLA-B40 in the elderly group (20% v 12%). Clearly the use of blood donors as controls can be criticised, but in the absence of a better control group, are frequently used in blood group and disease association studies.

The choice of non-demented chronic inpatients as a control group for the CMV study is also not ideal. This group had been in hospital for much longer than the study patients, and had presumably been more exposed to CMV infection which is known to be common in institutions. The possible effects of prolonged psychotropic drugs on the immune system and viral antibody titres of the controls, have also been ignored. However their selection as controls was thought to be better than having no

control group for the CMV study.

5) BLOOD GROUPING AND HLA TISSUE-TYPING

Technical errors can occur in blood grouping and tissue-typing if carried out by inexperienced technicians. Some of the difficulties which may arise in tissue-typing have been pointed out by Joysey and Wolf (462); cross-reactions may occur between specific HLA antigens and it is therefore advisable to use several antisera for their detection. Other sources of error include contamination of live lymphocyte suspensions on which the tissue-typing is carried out.

In this study, blood grouping and HLA-typing were performed by experienced technicians at the Leeds Regional Blood Transfusion Centre and three well authenticated antisera were used to detect HLA-B15. The increased frequency of HLA-B15 is unlikely to have arisen as the result of cross-reactions with HLA-B17, B5 and B35, to which some degree of cross-reactivity with B15 is well recognized, because the frequencies of these antigens in the patients did not differ significantly from those in the controls.

It is therefore unlikely that significant technical errors occurred, and any minor errors would probably be

randomly distributed, and not adversely affect the results.

6) ANALYSIS OF RESULTS

The statistical methodology commonly used in ABO and Rh blood group studies have been severely criticised by Weiner (515) and Manuila (516), and their criticisms are also valid for HLA studies.

False significant results can easily arise from errors in data collection and their statistical treatment, and the lower the number of patients included in a study, the higher the statistical sampling error which can occur. Data from other centres are commonly pooled for statistical purposes to overcome the difficulties of accumulating large numbers of patients at any one centre, but the logic for doing so has been questioned by Weiner (515).

In this study patients were tested for 20 HLA antigens, so that by chance, at least one antigen may be altered in frequency, either increased or decreased, at the 5% level of significance in the patients compared with the controls (473). One way of overcoming this difficulty is to multiply the P value obtained by the number of antigens studied to give a corrected P value (473). This procedure is rather conservative, and true HLA

associations with disease may be missed. The only definitive method of confirming an association is for other independent workers to find a similar association.

ABO BLOOD GROUP RESULTS

DISCUSSION1) ABO BLOOD GROUP RESULTS:

The ABO blood group frequencies of the patients do not deviate significantly from the controls. Blood groups AB and B are relatively underrepresented, and group A overrepresented in the PDAT group, but as the numbers of patients are small, sampling error may be a possible explanation for these findings.

The only study of ABO blood groups in PDAT patients which the writer is able to trace, found no obvious associations (188), although the frequency of blood group A was slightly raised as in this study.

Mourant, Kopec and Domaniewska-Sobczak (517) list 12 ABO blood group studies of senile dementia, although many of these can be criticised because of imprecise selection criteria and the inclusion of small numbers of patients. Several studies report an apparent association, but no consistent pattern is found. Normal ABO blood group distributions were found in 2 British studies of demented patients which included SDAT cases (518,519).

The failure of this study to find any significant association of ABO blood groups with AD, is therefore in agreement with the findings of most other studies.

RHESUS BLOOD GROUP RESULTS

2) RHESUS BLOOD GROUP RESULTS:

This study shows a significant association of the Rh negative blood group with SDAT, but clearly caution is necessary in the interpretation of this result, as relatively few patients were studied. However two other similar studies of SDAT patients are listed by Mourant, Kopec and Domaniewska-Sobczak (520), and both found an excess of rhesus negatives. Data from these and the present study can be combined using Woolf's method (521, 522) of statistical analysis to allow a more accurate assessment of the relative risk and statistical significance of the association of the rhesus negative blood group with SDAT to be made. Heterogeneity between the three centres can also be tested with Woolf's formula.

The results of this combined analysis are shown in table 23. The rhesus negative blood group is found to be significantly associated with SDAT (pooled $X^2 = 13.778$, dfl, $p = 0.0002$). There is no significant heterogeneity between the relative risk obtained in the individual studies, and it is therefore justifiable to combine the results of the three studies. The weighted estimated mean value of the relative risk is 1.9.

Masters (519) also found an excess of rhesus negatives among a group of elderly patients with "mixed organic states" which included SDAT.

However a similar association with PDAT would also be expected if both conditions share common genetic

factors. The failure to find an excess of rhesus negatives among the presenile patients in this study may mean that the SDAT result is spurious, but could also indicate that a different genotype is present in some SDAT patients, which is absent in the PDAT group. The failure of two other studies to demonstrate an association of the rhesus negative blood group with PDAT would support the latter hypothesis.

This association, if confirmed, could indicate a causal relationship, in that rhesus negatives are intrinsically more likely to develop SDAT. This could be due to the pleiotropic or multiple effects of the same gene. For example, one possible explanation for the association of thromboembolic diseases with blood group A is that the plasma antihaemophilic globulin is raised in group A persons relative to that in group O individuals (484).

Clarke (482) has also suggested that the increased susceptibility to disease could result from the direct immunological effect of the blood groups themselves. The immunological importance of the ABO blood groups for example, is shown by their influence on tissue survival in organ transplantation (523).

Blood groups could also influence disease predisposition by increasing susceptibility to infectious agents. This theory is supported by reports of significant associations between blood groups and some

human infectious diseases. (524-529).

Why humans of different blood types vary in their resistance to infections is unclear. Some bacteria and viruses are known to have blood group-like antigens in common with humans (530-535), and it is possible that disease susceptibility is partly determined by the presence of blood group antibodies. An invading pathogen with an A-like antigen which is partially neutralized by anti-A antibodies in people with blood group O, could for example, result in an increased association of the infection with blood group A individuals who lack anti-A antibodies.

However the association of the rhesus negative blood group with SDAT could also be indirect, and result from factors such as population stratification or linkage disequilibrium.

TABLE 23

The association of senile dementia of the Alzheimer type with the Rhesus negative blood group, using Woolf's method of statistical analysis. Data from Greece, Italy and Leeds

| Centre | AD Patients | | Controls | | $X^* = \frac{hK}{Hk}$ | $y = \text{Loge } X$ | W^{**} | W_y | $X^2 = \frac{W_y^2}{W}$ |
|--------------|-------------|------------|------------|------------|-----------------------|----------------------|----------|---------|-------------------------|
| | Rh- (h) | Rh+ (k) | Rh- (H) | Rh+ (K) | | | | | |
| Greece (520) | 6 | 33 | 556 | 3923 | 1.2829 | 0.2491 | 5.0226 | 1.2511 | 0.3119 |
| Italy (520) | 17 | 40 | 148 | 852 | 2.4466 | 0.8948 | 10.8933 | 9.7473 | 8.7223 |
| Leeds | 25 | 62 | 3771 | 16948 | 1.8122 | 0.5945 | 17.6991 | 10.5221 | 6.2549 |
| | | | | | | Sum | 33.615 | 21.5205 | 15.2891 |

211

*X = The relative incidence of SDAT in Rhesus negative patients compared to 1 in Rhesus positive patients

$$**W = \frac{1}{\frac{1}{h} + \frac{1}{k} + \frac{1}{H} + \frac{1}{K}}$$

The pooled $X^2 = \frac{(21.5205)^2}{33.615} = 13.7775$; Therefore $P = 0.0002$, for one degree of freedom.

The heterogeneity $X^2 = 15.2891 - 13.7775 = 1.5116$

With two degrees of freedom $0.5 > P > 0.25$. Therefore there is no apparent heterogeneity in the results of the three studies, which may therefore be combined.

HLA ANTIGEN

RESULTS

DISCUSSION3) HLA ANTIGEN RESULTS:

This study shows a significant association of HLA-B15 with PDAT and SDAT. Table 24 shows the estimated relative risk (RR) of developing AD when HLA-B15 is present for each of the patient groups in the study. The RR for the presenile group is 3, and 2.3 for the senile patients.

Five other HLA studies of AD patients have been reported (368,536-539):

Henschke, Bell and Cape (536) studied 34 patients with AD mainly of the SDAT type, for 38 HLA-A, -B, and -C antigens. Only the frequency of HLA-Cw3 was significantly increased over controls (38.2% v 21.6%, $P < 0.05$, RR 2.3), but the findings lost significance when correction was made for the number of antigens tested.

Cohen, Zeller, Eisdorfer and Walford (537) studied 25 patients with unspecified AD for 34 HLA-A, -B, -C and DRw antigens. Unfortunately the number of controls used in this study were not specified, and the results for only 3 HLA antigens were reported. The frequencies of HLA-B7 (36% v 18%), Cw3 (32% v 20%), and DRw4 (43% v 32%) were increased over controls.

Walford and Hodge (538) studied 55 patients suffering from PDAT and SDAT for 36 HLA antigens of the HLA-A, -B, -C and DR loci, and compared the frequencies obtained

with an unspecified number of controls. The frequency of HLA-B7 was found to be significantly increased over the controls (36.4% v 16.8%, uncorrected $P = 0.0001$).

Whalley et al (368) examined HLA-A and -B antigen frequencies in 14 patients with PDAT and 64 healthy controls, and reported no significant differences between the patients and controls.

Wilcox, Caspary and Behan (539) studied 10 PDAT patients who had developed the disease before the age of 60 for HLA-A and -B antigens, but no significant associations were found. They also studied 8 SDAT patients, but there is some doubt as to the correct diagnosis in this group. Apparently there was a previous history of alcoholism in some, and of a stroke in others.

These studies involved relatively few patients, and the RR would have to be large for any real HLA association with AD to show up significantly.

The reported association of AD with HLA-B7 was not confirmed in the present study. When the RR for HLA-B7 is calculated from each study (Table 25), it is found to be increased in 4 of the 6 studies, which may possibly indicate a real association.

As the HLA-C and -D locus antigens were not tested in this study, the reported association of AD with Cw3 and DR4 cannot be confirmed.

When the RR for HLA-B15 is calculated from each study (Table 27), it is found to be increased in 3 of the 5 studies. As Walford and Hodge did not include the

number of controls used in their study, their HLA findings cannot be combined with data from other centres for the purposes of statistical analysis. However as there is no apparent heterogeneity in the results, the HLA-B15 finding of the present study can be combined with the HLA-B15 findings of the other studies for which the number of controls are known, using Woolf's formula (521,522).

When all 4 results are combined (Table 26), the association of HLA-B15 with AD remains statistically significant (pooled $X^2 = 10.1538$, $P = 0.0015$), although the major contribution to the pooled X^2 has come from this study. The weighted estimated mean value of the RR for HLA-B15 is 1.73. However the failure to find an increased RR for this antigen in 2 studies indicates that on present evidence, it cannot be definitely concluded that HLA-B15 is associated with AD. Further studies involving larger numbers of patients are clearly necessary to confirm such an association.

AD may well be a genetically heterogeneous disorder with polygenic inheritance, and a number of disease susceptibility genes may predispose to its development. However if an association of HLA-B15 with PDAT and SDAT is confirmed, it would suggest that some patients with the presenile and senile forms of AD share a common genetic predisposition.

Theoretical mechanisms to explain the possible
association of HLA antigens and Alzheimer's disease:

Two main theories have been proposed to explain HLA and disease associations (446-450,453,463,467,471,540, 541), and both assume that viruses or other pathogenic agents may be implicated, either directly or indirectly, in the disease process.

1) The Immune Response Gene Hypothesis:

The most popular theory advanced to explain HLA and disease association proposes that the association occurs not as a direct effect of the HLA antigens themselves, but to the effects of closely linked disease susceptibility genes. There has been speculation that these disease susceptibility genes may in fact be immune response (Ir) genes, which normally control the capacity of the host to mount an immune response against foreign antigens such as viruses, but when defective may initiate disease through a pathological immune response.

There is no direct evidence for the presence of Ir genes in man, but it is deduced from work in mice and guinea pigs that they must exist in the major histocompatibility region in man. Ir genes are thought to map within the HLA region between the B and D loci, and be in linkage disequilibrium with specific alleles at the HLA loci (447,448). Therefore in an HLA-associated

disease, any HLA antigens which are coded for by HLA genes in linkage disequilibrium with Ir genes, will be increased in frequency. The closeness of the HLA-B and more recently recognized -D loci to the proposed Ir genes may explain why most HLA-associated diseases have their strongest associations with B and D-locus antigens.

Linkage disequilibrium also occurs between some of the HLA-A, -B, -C and -D antigens, and the HLA antigens A2, B15, B40, Cw3 and Dw4 are in linkage disequilibrium with each other (463,542-544). The relative risks of developing AD when these antigens are present in this and other HLA studies of AD are shown in Table 27. The RR for HLA-A2 is increased in all the studies, although not significantly. However when the HLA-A2 results are combined (excluding the Los Angeles results for which the number of controls are unknown) using Woolf's formula (Table 28), a statistically significant association with this antigen is found (pooled $X^2 = 4.2388$, $P = 0.04$).

Similarly the RR for HLA-B40 is also increased in all the studies, but the association is not statistically significant when the results are combined using Woolf's formula (Table 29).

The RR for HLA-Cw3 is also increased in the three studies which have examined this specificity.

If the associations of these antigens with AD are real, it might be expected that the primary HLA association of the disease would be with HLA-Dw4, as the D locus is thought to be the most closely linked of all the HLA

loci to the Ir genes. The associations with A2, B15, B40 and Cw3 would then be secondary. A similar association for example has been found in juvenile-onset diabetes which is primarily associated with HLA-Dw4 and secondarily associated with A2, B15, B40 and Cw3 (542). Also in multiple sclerosis, the initial associations with HLA-A3 and B7 were fairly weak, but more recent studies have found a closer association with HLA-Dw2, which is in linkage disequilibrium with HLA-A3 and B7 (451). However although the 2 studies which tested AD patients for the HLA-Dw4 antigen found the expected increase in frequency, the RR for this antigen is very small.

It is clear that if the associations of HLA-A2, B15, B40, Cw3 and Dw4 with AD are real, the relative risks for these antigens are small, and it is impossible to be sure which shows the strongest HLA association with the disease. Furthermore if the Ir gene theory is correct, the association of these HLA antigens with AD would be secondary to the effects of linkage disequilibrium with a closely linked Ir gene (or genes) which predisposes to AD.

Evidence in support of Ir genes has come from experimental work on mice. Murine studies have demonstrated that the immune responses to some synthetic and native antigens, and susceptibility to certain viruses such as the Gross virus and lymphocytic-choriomeningitis virus, are partly controlled by genes which lie within the H-2 system (466,453,457,541). In the absence of direct experimentation, evidence in favour of HLA-associated Ir

genes in man have been less easy to obtain, and in fact there is no direct evidence for their presence. One source of evidence in favour has come from some human studies of skin test reactions to natural allergens (545). Further support for the presence of HLA-associated Ir genes has come from Buckley and Roseman (546) who found that antibody levels to common viruses are frequently HLA-associated within Amish families.

However these and similar studies can be criticized on several counts. They have been retrospective, and no consideration has been given to the individual's degree of previous exposure. Also in some cases, initial positive findings have not been confirmed.

Associations between autoantibodies and HLA antigens have also been reported in humans (547).

There is also evidence supporting a direct association of an HLA-linked Ir gene with a specific immune response, and severity of disease. Vladutiu and Rose (548) have shown that the degree of susceptibility to murine autoimmune thyroiditis, and thyroid autoantibody response is related to different alleles of the H-2 locus, and that the severity of autoimmune thyroid damage is correlated with the autoantibody response.

Human evidence is less convincing however. Levine, Stember and Fotino (549) reported that clinical ragweed hayfever and IgE antibody production specific for antigen E (the major purified protein antigen from ragweed pollen extract) was significantly associated with HLA haplotypes

in successive generations of seven families which they studied, although other workers have failed to replicate these results.

The immune response theory is attractive, as many HLA associated diseases such as juvenile-onset diabetes and multiple sclerosis are thought to have an immune aetiology and viral involvement is suspected. There has been speculation that the destruction of pancreatic β cells in juvenile-onset diabetes results from an HLA-linked genetically controlled immune response to viruses (542). This is supported by the findings of Cudworth and Festenstein (542) that HLA-B8 and B15 positive diabetics have higher neutralizing antibody titres to Coxsackie virus (B1-4 variants) than have diabetics without these antigens.

Similarly it has been suggested that multiple sclerosis (MS) may be the consequence of a genetically controlled immune response which is initially provoked by a common virus (550,551). MS patients have significantly higher levels of measles antibody compared with controls (551), but the findings of Jersild et al (552) that serum measles antibody titres are significantly increased in MS patients with HLA A3 and B7, compared to MS patients with other tissue types, has not been entirely confirmed (550).

The possible association of HLA-B15 with an immune response trait to CMV is therefore of interest, and raises the possibility that Alzheimer's disease could result from

an abnormal immune response mounted by the host against CMV infected neurons.

2) Direct effect of the HLA antigens:

This theory proposes that HLA and disease associations occur not through linkage disequilibrium with Ir genes, but because of the direct participation of specific HLA antigens themselves in the disease process. There are three main ways in which this might be achieved:

a) Molecular mimicry (cross tolerance) hypothesis:

This hypothesis assumes that a pathogenic agent which directly causes an HLA-associated disease shares common antigenic determinants with specific HLA antigen(s). An individual possessing this particular HLA antigen would then be less likely to recognize the pathogen as foreign and mount a successful immune response against it. They would therefore be more susceptible to infection, infection would be more likely to persist, and disease ensue. It has been suggested for example that the association of HLA-B27 and ankylosing spondylitis may be due to cross reactivity between a klebsiella serotype and a gene product closely associated with HLA-B27 (553).

b) Virus receptor hypothesis:

This hypothesis assumes that HLA antigens act as cell membrane receptors for disease-producing viruses.

Evidence in support of the virus receptor theory has come from studies which have demonstrated that human influenza virus-immune cytotoxic T lymphocytes recognize viral antigens in conjunction with self antigens that are highly associated with the serologically defined HLA-A and -B specificities (554).

c) Theory of modified self:

According to this theory, viruses change the immune characteristics of infected cells, and disease results from an ensuing autoimmune response. This cellular modification could be achieved in various ways. For example new antigenic complexes could result from the combination of viruses with HLA genes or antigens, or the genetic apparatus regulating expression of HLA antigens could become derepressed following viral infection.

The evidence that viruses can alter HLA expression is far from conclusive, but Pellegrino et al (555) reported that SV40 virus - transformed human cells expressed an HLA specificity (B5) not present on the surfaces of uninfected cells.

3) Other theories:

It is most unlikely that all HLA and disease associations can be explained by any one theory, and other mechanisms have been proposed (453,540).

Some HLA-associated diseases may result from defects in enzyme systems closely linked to the major histocompatibility system. For example, some components of the complement system are coded for by genes in the HLA region, and complement deficiencies could perhaps cause disease by interfering with complement-dependent immune responses (453,468). Other HLA-associated disorders may result from the actions of genes at different loci within the major histocompatibility system which themselves have no relevance to the functions of this system.

TABLE 24

Estimate of relative risk of developing Alzheimer's disease when HLA-B15 is present

| PDAT Males (n = 13) | PDAT Females (n = 24) | PDAT Males and Females (n = 37) | SDAT Males (n = 37) | SDAT Females (n = 50) | SDAT Males and Females (n = 87) | PDAT + SDAT Males (n = 50) | PDAT + SDAT Females (n = 74) | PDAT + SDAT Males and Females (n = 124) |
|---|-----------------------------|---|---------------------------|-----------------------------|---|-------------------------------------|---------------------------------------|--|
| % of patients who are 23.08 HLA-B15 positive* | 37.50 | 32.43 | 29.73 | 24.00 | 26.44 | 28.00 | 28.38 | 28.20 |
| Relative risk for 1.9 HLA-B15 | 3.8 | 3.0 | 2.7 | 2.0 | 2.3 | 2.4 | 2.5 | 2.5 |

*% of 458 controls HLA-B15 positive = 13.75%

TABLE 25

The relative risk of developing Alzheimer's disease when HLA-B7 is present. Data from Canada, United States of America, Newcastle and Glasgow, Edinburgh and Leeds.

| Centre | No. Patients studied | RR for HLA-B7 |
|----------------------------------|-------------------------|------------------|
| Canada (536) | 34 | 1.6 |
| Seattle and Los Angeles (537) | 25 | 2.6 |
| Los Angeles (538) | 55 | 2.8 |
| Newcastle and Glasgow (539) | 18 | 1.3 |
| Edinburgh (368) | 14 | 0.8 |
| Leeds | 124 | 0.9 |

TABLE 26

The association of Alzheimer's disease with HLA antigen B15, using Woolf's method of statistical analysis. Data from Canada, Newcastle and Glasgow, Edinburgh and Leeds

| Centre | AD Patients | | Controls | | X | y | W | Wy | X ² = Wy ² |
|-----------------------------------|-------------|-------------|------------------|-------------|--------|--------|---------|---------|----------------------------------|
| | B15+ (h) | B15- (k) | B15+ B15- (H) | B15- (K) | | | | | |
| Canada (536) | 5 | 29 | 24 | 215 | 1.5445 | 0.4349 | 3.5599 | 1.5482 | 0.6732 |
| Newcastle and Glasgow (539) | 0 | 18 | 29 | 313 | 0 | 0 | 10.7181 | 0 | 0 |
| Edinburgh (368) | 4 | 10 | 11 | 53 | 1.9273 | 0.6560 | 2.1749 | 1.4267 | 0.9359 |
| Leeds | 35 | 89 | 63 | 395 | 2.4657 | 0.9026 | 17.1821 | 15.5086 | 13.9983 |
| | | | | | Sum | | 33.6350 | 18.4835 | 15.6074 |

226

The pooled $X^2 = \frac{(18.4835)^2}{33.635} = 10.1573$; Therefore $P = 0.0015$, for one degree of freedom .

The heterogeneity $X^2 = 15.6074 - 10.1573 = 5.4501$

With three degrees of freedom $0.2 > P > 0.1$. Therefore there is no apparent heterogeneity in the results of the four studies, which may therefore be combined .

TABLE 27

The relative risk of developing Alzheimer's disease when HLA antigens A2, B15, B40, Cw3 and Dw4 are present - data from Canada, United States of America, Newcastle and Glasgow, Edinburgh and Leeds

| Centre | No patients studied | Relative Risk | | | |
|-------------------------------|---------------------|---------------|-----|-----|-----|
| | | A2 | B15 | B40 | Dw4 |
| Canada (536) | 34 | 1.1 | 1.6 | 1.4 | - |
| Seattle and Los Angeles (537) | 25 | - | - | - | 1.6 |
| Los Angeles (538) | 55 | 1.5 | 0.2 | 1.6 | 1.3 |
| Newcastle and Glasgow (539) | 18 | 1.4 | 0 | 2.1 | - |
| Edinburgh (368) | 14 | 1.6 | 1.9 | 1.6 | - |
| Leeds | 124 | 1.5 | 2.5 | 1.5 | - |

TABLE 28

The association of Alzheimer's disease with HLA antigen A2, using Woolf's method of statistical analysis. Data from Canada, Newcastle and Glasgow, Edinburgh and Leeds

| Centre | AD Patients | | Controls | | X | y | W | Wy | X ² = Wy ² |
|-----------------------------------|-------------|------------|------------|------------|--------|--------|---------|---------|----------------------------------|
| | A2+ (h) | A2- (k) | A2+ (H) | A2- (K) | | | | | |
| Canada (536) | 16 | 18 | 107 | 132 | 1.0966 | 0.0926 | 7.4019 | 0.6854 | 0.0635 |
| Newcastle and Glasgow (539) | 10 | 8 | 163 | 179 | 1.3727 | 0.3171 | 4.2248 | 1.3397 | 0.4250 |
| Edinburgh (368) | 8 | 6 | 29 | 35 | 1.6092 | 0.4755 | 2.8185 | 1.3402 | 0.6373 |
| Leeds | 73 | 51 | 225 | 233 | 1.4823 | 0.3935 | 23.8095 | 9.3690 | 3.6857 |
| | | | | | | Sum | 38.2547 | 12.7343 | 4.8115 |

The pooled $X^2 = \frac{(12.7343)^2}{38.2547} = 4.239$; Therefore $P = 0.04$, for one degree of freedom .

The heterogeneity $X^2 = 4.8115 - 4.239 = 0.5725$

With three degrees of freedom $P > 0.5$. Therefore there is no apparent heterogeneity in the results of the four studies, which may therefore be combined.

TABLE 29

The association of Alzheimer's disease with HLA antigen B40, using Woolf's method of statistical analysis. Data from Canada, Newcastle and Glasgow, Edinburgh and Leeds

| Centre | AD Patients | | Controls | | X | y | W | Wy | X ² = Wy ² |
|-----------------------------------|-------------|-------------|------------------|-------------|--------|--------|---------|--------|----------------------------------|
| | B40+ (h) | B40- (k) | B40+ B40- (H) | B40- (K) | | | | | |
| Canada (536) | 7 | 27 | 38 | 201 | 1.3714 | 0.3155 | 4.7371 | 1.4946 | 0.4713 |
| Newcastle and Glasgow (539) | 3 | 15 | 30 | 312 | 2.08 | 0.7325 | 2.2909 | 1.6781 | 1.2293 |
| Edinburgh (368) | 2 | 12 | 6 | 58 | 1.6111 | 0.4769 | 1.3034 | 0.6216 | 0.2964 |
| Leeds | 17 | 107 | 44 | 414 | 1.4949 | 0.4023 | 10.7066 | 4.3073 | 1.7334 |
| | | | | | | Sum | 19.038 | 8.1016 | 3.7304 |

The pooled $X^2 = \frac{(8.1016)^2}{19.038} = 3.4476$; Therefore $P = 0.0643$, for one degree of freedom.

The heterogeneity $X^2 = 3.7304 - 3.4476 = 0.2828$

With three degrees of freedom, $P > 0.5$. Therefore there is no apparent heterogeneity in the results of the four studies, which may therefore be combined.

HLA-B15 AND
CLINICAL CHARACTERISTICS

DISCUSSION4) HLA-B15 AND CLINICAL CHARACTERISTICS:

The finding that the male and female patients have a similarly increased frequency of HLA-B15 is interesting. As women have a higher prevalence of AD, it might be expected that men who develop the disease have a greater genetic predisposition, and hence show a higher frequency of genetic markers of disease susceptibility genes such as HLA-B15.

This study found no evidence that the possession of HLA-B15 favours the early development of AD, or influences its duration. The association of insulin-dependent diabetes and HLA antigens is strongest in early onset cases (474), and it is interesting to observe that the highest proportion of HLA-B15 positive patients found in this study were those who developed AD between the ages of 50-54 (55.6%). This finding hints at a possible association of HLA-B15 with early disease onset, but as the number of patients included in this particular age range was small, no firm conclusions can be drawn.

RESULTS OF FAMILY
STUDIES

DISCUSSION5) FAMILY STUDIES:

No histological findings were available to confirm the diagnosis of AD in the secondary cases, and caution is therefore necessary in the interpretation of the results of the family studies. However, the occurrence of dementia in 9.7% of the first degree relatives of patients with AD, is further evidence that genetic factors are important in the aetiology of this disorder. The occurrence of cases of PDAT and SDAT within the same families also suggests that they share a common genetic predisposition.

The failure to find any significant difference in the frequency of AD among the relatives of the 10 HLA-B15 positive and 10 HLA-B15 negative patients matched for sex and age at onset of disease is to be expected if the association of HLA-B15 and AD is due to linkage disequilibrium with a gene predisposing to AD.

The finding of a significantly increased frequency of AD when the 24 B15 positive patients were compared with the 10 B15 negative patients must be interpreted with great caution, as far fewer B15 negatives were studied and statistical sampling error could therefore easily have arisen.

If susceptibility to disease depends upon the inheritance of an HLA-linked gene, then it would be expected

that affected siblings will always be identical for one or both haplotypes. In the present study only 2 secondary cases of dementia were alive and available for tissue typing. Patient number 62 shared 2 HLA antigens (A2, B15) in common with an affected younger brother, and patient number 69 shared identical antigens (A1, 9, B12, 15) with an affected older sister. However no conclusions can be drawn about possible linkage of AD susceptibility genes to HLA genes from this study, as even on the assumption that there is no linkage, affected sibpairs would have a 25% chance of being HLA identical, and a 50% chance of sharing one haplotype. Evidence of HLA linkage in AD must await larger studies of families with 2 or more affected relatives still alive and available for tissue typing.

THE RELATIONSHIP OF
ALZHEIMER'S DISEASE TO THE
MYELOPROLIFERATIVE DISORDERS
AND DOWN'S SYNDROME

DISCUSSION6) THE RELATIONSHIP OF ALZHEIMER'S DISEASE TO THE
MYELOPROLIFERATIVE DISORDERS AND DOWN'S SYNDROME:

The increased incidence of myeloproliferative disorders and Down's syndrome among the relatives of PDAT cases (102,103) suggests that these conditions may share a common genetic predisposition, and it is of interest that 2 relatives with a possible history of leukaemia were found in this study.

If a common genetic factor were linked to the HLA region, it might be expected that these disorders would show a common association with the HLA antigens which are associated with AD.

The earliest reports suggested that Hodgkin's disease was associated with the compound antigen 4C, which was later split into HLA-B5, B35, B18 and B15 (556). Subsequent studies have suggested a weak association with HLA-A1, B5, B8 and B18 (556).

Acute lymphatic leukaemia is weakly associated with HLA-A2 (472,473,556), (RR 1.3), and its possession may also increase survival in the disease (556), perhaps because patients with this antigen may have a lower incidence of blood transfusion reactions (557). Harris et al (558) also found a tendency for HLA-B15 (and A2, and A3) to increase progressively with survival in

patients with acute myelogenous leukaemia.

In the only reported HLA study of Down's syndrome, Segal et al (559) found no significant HLA associations in 76 patients with this disorder, although the RR for HLA-B15 was increased (1.6).

These results are interesting in view of the possible association of HLA-A2 and HLA-B15 with AD, but they do not allow the conclusion that the myeloproliferative disorders, Down's syndrome, and AD share a common genetic predisposition linked to the HLA region.

CMV ANTIBODYRESULTS

DISCUSSION

7) CMV ANTIBODY RESULTS

Although AD patients have a higher prevalence of CMV antibodies than the controls, the difference is not significant. This study therefore fails to confirm the findings of Lycke, Norrby and Roos (366) in Sweden, although the two studies are not strictly comparable. The Swedish study included some cases of multi-infarct dementia, and as the patients were older than the controls, age difference may have been partly responsible for the higher prevalence of CMV antibodies in the demented group. A recent Edinburgh study (368) also found no increased prevalence of CMV antibodies in 14 PDAT patients.

No significant differences are found between HLA-B15 positive and HLA-B15 negative patients in the prevalence of CMV antibodies. This study therefore fails to confirm the results of Pereira, James and Stern (560) who found that a significantly increased number of normal HLA-B15 positive individuals had CMV antibodies compared with normal HLA-B15 negative people.

Although the geometric mean antibody titre is higher in the patients than controls, the difference is not significant. However the mean geometric titre is significantly higher in the HLA-B15 positives than the

HLA-B15 negative patients or the controls. Although the Edinburgh study (368) found that no conventional virus appeared to be associated with any particular HLA antigen, it is interesting to note that of the 3 patients with CMV antibodies in the study, two were HLA-B15 positive.

The finding of an increased CMV antibody titre in the HLA-B15 positives however raises a theoretical objection to the hypothesis that the association of B15 with AD results from linkage disequilibrium with a disease susceptibility gene. If this theory is correct, no difference in CMV antibody titre would be expected between B15 positives and B15 negatives. Such a difference in CMV antibody titre, if real, would imply genetic heterogeneity.

There are several possible explanations for the higher mean CMV antibody titres in the HLA-B15 positives found in this study. It could indicate that HLA-B15 is an immunological marker for a heightened antibody response to CMV, which would partly explain the results of the Swedish study (366) if HLA-B15 is associated with AD. Alternatively this heightened antibody response could also reflect a selective impairment of cell-mediated immunity to the CMV in HLA-B15 positive patients. An association between CMV susceptibility and the murine major histocompatibility complex has also been reported (561).

A further possible explanation is that HLA-B15 phenotypes are high antibody responders to all forms of antigenic stimulation. However although an association with HLA-B15 and anti-mitochondrial antibody has been reported (547), the failure of Pereira, James and Stern (560) to find any relationship between this antigen and common virus antibodies, apart from CMV, is against such a hypothesis.

The possible association of HLA-B15 with an immune response trait to CMV infection may be an epiphenomenon and have no relevance to the aetiology of AD. However it could also indicate that the CMV may have a causative role in some cases of the disease, either as the direct result of persistent infection, or through the initiation of an aberrant immune response within the brain. The failure to find elevated serum CMV antibodies in the HLA-B15 negative patients does not necessarily preclude such a possibility, for although perhaps initiating the disease, the CMV may no longer be present when dementia becomes apparent, or may be present in a defective form. In the following discussion the various mechanisms by which persistent CMV infection could be of aetiological importance in AD will be considered.

Possible mechanisms by which Alzheimer's disease could result from persistent CMV infection:

There are two main ways in which persistent CMV infection could play an aetiological role in AD.

1) Direct effect of CMV on neurons:

a) Direct cytopathic effect:

Reactivation and replication of latent CMV within the brain as the result of an age-related decline in cell-mediated immunity, could destroy infected neurons by a cytopathic effect, and a resultant acute inflammatory response against the neuronal breakdown products. However, although much of the tissue damage in congenital CMV infection and herpesvirus encephalitides is thought to be produced in this manner, it is very unlikely that the neuropathological features of Alzheimer's disease could result from such a process. The failure to grow conventional CMV in cell cultures from AD brain specimens is also against the concept of a direct involvement of an acute CMV infection in the pathogenesis of this disorder.

b) Interference with neuronal metabolism:

Gajdusek (562) has speculated that if metabolic or biochemical factors of cell ageing with enzyme exhaustion underlie the formation of senile plaques and neurofibrillary tangles in the ageing brain, similar but premature

cellular metabolic changes might be induced by slow, latent or modified defective viruses.

It is therefore possible that latent or modified CMV could persist in an integrated state within the neurons, despite high levels of CMV antibody which cannot penetrate cells (563), with adverse effects on neuronal metabolism.

2) Effect of CMV on the immune system:

The evidence which suggests that the pathogenesis of AD is mediated through immune mechanisms has already been considered. There are a variety of ways in which the CMV could initiate an abnormal immune response which could result in AD:

a) Immune complex disease:

Immune complex disease results when antigen and host antibody aggregates are formed and deposited into tissues over a long period of time. Immune complexes (IC) can be formed in chronic viral infections (564-566) in which viral antigens and host antibody are being constantly produced.

Infection of newborn mice with lymphocytic choriomeningitis (LCM) virus for example, results in a persistent infection with the formation of immune complexes which are deposited in kidneys, blood vessels

and the choroid plexus of the CNS, but disease is not manifested until 7-10 months later (564,567,568). The incubation period of congenital and neonatal LCM infection is more than half the normal expected life span of mice, and the disease has been likened to premature senescence (568).

Transient immune complex formation has been reported in acute CMV infections, which disappear following clinical recovery (569-571).

It is unknown whether a chronic CMV infection resulting from the reactivation of the virus from a latent state could induce prolonged immune complex formation. However immune complexes have been detected in the renal glomeruli of mice with chronic CMV infection (572), and have also been found in patients with clinical and subclinical congenital CMV infections during the first year of life (573).

The pathology of Aleutian disease of mink, which is caused by a slow virus, is thought to be mediated by immune complexes (574), but at present there is little evidence to suggest that Alzheimer's disease is similarly mediated. However Wisniewski and Terry (575) have speculated that in Alzheimer's disease, changes in the permeability of the blood-brain barrier could result in toxic antigen-antibody complexes leaking out of the blood stream to cause neuritic degeneration and senile plaque formation.

b) Autoimmunity:

Transient immunological abnormalities and autoimmune

phenomena, including autoimmune haemolytic anaemia, rheumatoid factor, antinuclear antibodies, cold agglutinins, cryoglobulins and positive Coomb's tests, have been observed in acute congenital human (411,412,569-571,576) and chronic murine (572) CMV infections. It is unclear whether these autoantibodies have any primary pathogenic significance or are merely secondary to tissue damage produced by CMV infection. However their presence raises the possibility that CMV infection could play a role in the pathogenesis of Alzheimer's disease by the initiation of an antibody or cell-mediated autoimmune reaction against virus-infected neurons. This could be achieved in a variety of ways:

1) Release of sequestered antigens:

Replication of CMV with subsequent neuronal destruction, could result in the release of intracellular components such as DNA and mitochondria, and conceivably an autoimmune response could be provoked against these newly exposed antigens. The reported association of HLA-B15 and enhanced autoantibody response to mitochondria (547) is interesting in this context, and raises the possibility that CMV infection could accelerate this process.

2) Virus-induced cell membrane alterations:

Herpesviruses can induce new virus-specific antigens on the surfaces of infected cells (566,577). Human

fibroblasts have also been shown to develop a new HLA antigen specificity after transformation with the virus SV40 (555).

3) Cross-reactivity between viral and host antigens:

CMV infection could provoke an autoimmune response if the virus and the host shared common antigenic determinants. Viruses may incorporate host cell antigens into their own cell walls as they enter or leave a cell, and also during viral assembly. In this situation an antiviral immune response could also be directed against host cells as well as the virus.

4) Infection of lymphocytes and macrophages:

Human CMV can infect and replicate in lymphocytes and macrophages, which could lead to a genetic alteration and function of these cells, and the initiation of an autoimmune response. For example, the loss of suppressor T cell control over B cells, or stimulation of autoantibody-producing B cells following CMV infection, could trigger such a process.

c) Immune suppression:

If the decline in immunity with increasing age predisposes to the development of Alzheimer's disease, CMV could be an important aetiological factor because of their immunosuppressive potential.

In mice, CMV infection causes depression of primary

and secondary humoral immune responses (578), and cell-mediated immunity to other antigens (579), and a significant decrease in the percentage of peripheral blood T cells has been reported in children congenitally infected with CMV (392,580). The immunosuppressive effect of murine CMV may be mediated by interaction of the virus with individual subpopulations of lymphocytes such as suppressor T cells, or macrophages (578), and similar mechanisms may be operating in human CMV infections. Macrophages are often found within senile plaques, and Scheinberg and Cathcart (213) have postulated that macrophage activation represents the initial step in the genesis of amyloid formation.

Immunosuppression can accelerate amyloid formation in experimental animals, and the report of Sim and Smith (581) that the clinical state of two patients with Alzheimer's disease actually deteriorated following treatment with cortisone and ACTH, is of some interest in this context.

d) Suppression of interferon:

Interferon is an antiviral glycoprotein produced by cells already infected with virus, which prevents viral replication in uninfected cells by inhibiting viral transcription and/or translation.

Murine CMV has been shown to inhibit interferon responses, and to have a synergistic effect on Newcastle disease virus in mice (582). It is therefore possible that human CMV could play a role in the development of

AD through their ability to suppress interferon responses, or through synergistic interaction with other viruses which are directly responsible for the initiation of the disease.

CREUTZFELDT-JAKOB
DISEASE IN AN HLA-B15
POSITIVE 15 YEAR
OLD GIRL

DISCUSSION8) CREUTZFELDT-JAKOB DISEASE IN AN HLA-B15
POSITIVE 15 YEAR OLD GIRL:

The very young age at onset of CJD in the patient D.H., and her high antibody titre to BK virus are worthy of note. However as Dr. A. Gay will be reporting this case in detail in the future, the writer's comments will be confined to the HLA findings, and the possible relationship of CJD to AD.

There are several features of CJD and AD which suggests a link between these two disorders. Their clinical findings can be very similar (583,584), and the stellate amyloid plaques which are common in AD are also found in about 10% of brains with CJD (312,328,585). The pathological changes of both CJD and AD have also been found in the same brain (329). The reports of the occurrence of AD and CJD in different members of the same family (69) is further support for an association between these two disorders.

Only about 6% of Africans are HLA-B15 positive (455), so that the possession of this antigen by the patient D.H. raises the possibility that CJD and AD share a common genetic predisposition, and perhaps even a common infectious aetiology.

A study (337) of HLA antigens in 25 members of a

Finnish family in which CJD was diagnosed in 8 cases, revealed no obvious linkage of the disease with a single haplotype, but at least 7 out of the 8 patients apparently showed the HLA antigens A28 and B8. Further studies of the sporadic cases of CJD are indicated to see if the disease is associated with any particular HLA antigen. However as the disorder is rare, it would be difficult to accumulate sufficient numbers of patients at any one centre.

PDAT IN ONE OF
MONOZYGOTIC TWIN SISTERS

DISCUSSION9) PDAT IN ONE OF MONOZYGOTIC TWIN SISTERS:

The occurrence of PDAT in only one of identical twins argues strongly that non-genetic factors are aetiologically important in some cases of AD. Three other cases of PDAT occurring in monozygotic twins have been reported:

Davidson and Robertson (586) described a woman with acne rosacea who started to dement at the age of 50, and who died aged 69, the post-mortem confirming the diagnosis of PDAT. The patient and her twin sister, who was unaffected, were considered to be identical, although no laboratory investigations were done to prove uniovularity. The affected twin had suffered from severe influenza with delirium and fever at the age of 38 during the 1918-19 epidemic.

Hunter, Dayan and Wilson (587) reported a second pair of female monozygotic twins with laboratory proof of uniovularity, who were apparently discordant for PDAT. Their patient died aged 64 after a dementing illness lasting 15 years, and at post-mortem the brain showed the typical features of AD. Her twin sister was unaffected.

Sharman et al (588) described PDAT in identical twin brothers. The disease was confirmed histologically in both, but no special genetic studies were carried out on the twins to prove monozygosity. Both brothers developed

the disease insidiously at the age of 34, and died 4 years later. Their mother had also developed a dementing illness at the age of 37, and died 5 years later. At post-mortem, her brain was found to be atrophied, but no histological examination was performed.

The affected twins in these reported cases have some features in common with the present case. In 3 of the 4 cases, twitching movements of the limbs were observed during the disease. Similar muscular twitchings in AD have been described by Jacob (589). In addition, all the discordant pairs were women, and their lengths of illness were unusually long.

The discordance for PDAT observed in 3 sets of identical twins suggests that environmental factors were operating in these cases, and clearly points to the need for further research in this area. However discordance does not prove that genetic factors are unimportant in PDAT, as they may act by increasing susceptibility to the environmental agents which are directly responsible for the disease.

CONCLUSIONS

CONCLUSIONS:

The following main conclusions are drawn from the observations of this study:

1) ABO blood groups do not appear to influence susceptibility to AD.

2) Persons who are rhesus negative may have a slightly increased risk of developing SDAT compared with rhesus positives. The failure to find a similar association in the PDAT group of patients may be the result of a statistical sampling error due to the small number of patients studied, but may also indicate that AD is a genetically heterogeneous disorder.

3) Persons who are HLA-B15 positive may be more predisposed to PDAT and SDAT than those without this tissue type.

4) The possible association of HLA-A2, B15, B40, Cw3 and Dw4 with AD may be secondary to the effects of linkage disequilibrium with a closely linked immune response gene predisposing to the disease.

5) The possible association of HLA-B15 with PDAT and SDAT supports the hypothesis that some patients with the presenile and senile forms of AD share a common

genetic predisposition.

6) The occurrence of cases of PDAT and SDAT within the same families is evidence that genetic factors are implicated in these disorders, and is further support for a common genetic predisposition.

7) The possession of HLA-B15 does not appear to favour the early development of AD or influence its duration.

8) AD patients who are HLA-B15 positive do not appear to have a stronger family history of AD than patients without this tissue type.

9) The occurrence of PDAT in only one of identical twins suggests that environmental factors are aetiologically important in some cases of the disease.

10) The finding that AD patients have similar CMV antibody titres to non-demented controls of a similar age, and the failure to detect conventional CMV activity in cell cultures, argues against a direct causal role for these viruses in most cases of AD.

11) The finding that HLA-B15 positive AD patients have higher mean antibody titres to CMV than AD patients with other tissue types, suggests that HLA-B15 is an

immunological marker for a heightened antibody response to these viruses, although may also reflect a defect in cellular immunity. Further studies of cellular and humoral immunity to CMV in AD patients are warranted.

12) The possible association of HLA-B15 with an immune response trait to CMV raises the possibility that these viruses may play an indirect role in the aetiology of AD in the HLA-B15 positive patients, perhaps through the initiation of an abnormal immune response directed against the brain.

APPENDICES

APPENDIX 1.

Details of the tissue-typing technique used at the Leeds
Regional Blood Transfusion Centre

1) Preparation of lymphocyte suspension:

(a) Approximately 3 ml quantities of heparinised blood is carefully layered onto equal amounts of Ficoll - Triosil mixture in labelled Rh tubes using a clean pasteur pipette.

Ficoll - Triosil mixture: 10 parts of 34% Triosil + 26 parts of 9% Ficoll mixed, SG adjusted to 1.076.

(b) Spin tubes at 2,000 rpm for 20 minutes (MSE) and then carefully remove the lymphocyte/platelet layer, with a clean pasteur pipette into labelled plastic centrifuge tubes. Fill tubes with Hank's balanced salt solution (HBSS).

(c) Wash lymphocytes 3 times in HBSS at 1,000 rpm for 5 minutes (MSE) discarding the supernatant and gently resuspending the lymphocyte button.

Finally resuspend the lymphocyte button in HBSS to give approximately $2 \times 10^6/\text{ml}$ concentration.

APPENDIX 1 - continued

2) Tissue-typing procedure:

Both National and Leeds typing plates are loaded with 1 μ l of serum.

(a) Add 1 μ l of lymphocyte suspension at 2×10^6 /ml to each sera well, wiping the pipette needle carefully between each well and ensure that all sera and cells are mixed together. Incubate the typing plate at room temperature for 30 minutes.

(b) Add 5 μ l of rabbit complement to each well, carefully wiping the pipette needle between each well, and incubate at room temperature for a further 60 minutes.

(c) Add 2 μ l of 5% Eosin (4°C) to each well, leave at room temperature for 10 minutes, add 2 μ l of formaldehyde solution (4°C) and read using inverted, phase contrast microscopy. The percentage of dead cells should be assessed as accurately as possible in 10% stages from negative to 100%, all positive reactions being noted.

APPENDIX 2

The HLA specificities and number
of antisera used in HLA-typing

| <u>Specificity</u> | <u>No. of</u> |
|--------------------|-----------------|
| <u>HLA-A</u> | <u>antisera</u> |
| 1 | 3 |
| 2 | 5 |
| 3 | 4 |
| 9 | 3 |
| 10 | 3 |
| 11 | 4 |
| 28 | 3 |

APPENDIX 2 - continued

The HLA specificities and number
of antisera used in HLA-typing

| <u>Specificity</u> | <u>No. of</u> |
|--------------------|-----------------|
| <u>HLA-B</u> | <u>antisera</u> |
| 5 | 5 |
| 7 | 4 |
| 8 | 3 |
| 12 | 3 |
| 13 | 3 |
| 14 | 3 |
| 17 | 3 |
| 27 | 3 |
| 35 | 3 |
| 40 | 4 |
| 15 | 3 |
| 18 | 3 |
| 22 | 3 |

REFERENCES

REFERENCES

- 1) Newton, R.D. (1948). The identity of Alzheimer's disease and senile dementia and their relationship to senility. *Journal of Mental Science*, 94, 225.
- 2) McMenemey, W.H. (1963). Alzheimer's disease: problems concerning its concept and nature. *Acta Neurologica Scandinavica*, 39, 369.
- 3) Lauter, H., and Meyer, J.E. (1968). Clinical and nosological concepts of senile dementia. In *Senile Dementia*, ed., Muller, C., and Ciompi, L. Bern: Hans Huber Publishers, p. 13.
- 4) Constantinidis, J. (1978). Is Alzheimer's disease a major form of senile dementia? Clinical, anatomical and genetic data. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 15.
- 5) McDonald, C. (1969). Clinical heterogeneity in senile dementia. *British Journal of Psychiatry*, 115, 267.
- 6) Sourander, P., and Sjögren, H. (1970). The concept of Alzheimer's disease and its clinical implications. In *Alzheimer's Disease and Related Conditions*, ed., Wolstenholme, G.E.W., and O'Connor, M. London: J. and A. Churchill, p.11.

- 7) Op den Velde, W., and Stam, F.C. (1973). Haptoglobin types in Alzheimer's disease and senile dementia. *British Journal of Psychiatry*, 122, 331.
- 8) Stam, F.C., and Op den Velde, W. (1978). Haptoglobin types in Alzheimer's disease and senile dementia. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 279.
- 9) Katzman, R. (1976). The prevalence and malignancy of Alzheimer's disease. A major killer. *Archives of Neurology*, 33, 217.
- 10) Slater, E., and Roth, M. (1969). In *Clinical Psychiatry*, (3rd ed). London: Bailliere, Tindall and Cassell, p. 615.
- 11) Lishman, W.A. (1978). In *Organic Psychiatry*, (1st ed.). Oxford, London, Edinburgh: Blackwell Scientific Publications, p. 540.
- 12) Jervis, G.A. (1956). The Presenile Dementias. In *Mental Disorders in Later Life*, ed., Kaplan, O.J. Stanford University Press, p. 262.
- 13) Terry, R.D. (1976). Dementia. A brief and selective review. *Archives of Neurology*, 33, 1.

- 14) Sjögren, T., Sjögren, H., and Lindgren, A.G.H. (1952). Morbus Alzheimer and Morbus Pick. A genetic, clinical and patho-anatomical study. Acta Psychiatrica Scandinavica, Suppl. 82.
- 15) Roth, M. (1955). The natural history of mental disorder in old age. The Journal of Mental Science, 101, 281.
- 16) Roth, M. (1978). Epidemiological studies. In Alzheimer's Disease: Senile Dementia and Related Disorders, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 337.
- 17) Kay, D.W.K., Beamish, P., and Roth, M. (1964). Old age mental disorders in Newcastle upon Tyne. Part I: A study of prevalence. British Journal of Psychiatry, 110, 146.
- 18) Nielsen, J. (1962). Geronto-psychiatric period-prevalence investigation in a geographically delimited population. Acta Psychiatrica Scandinavica, 38, 307.
- 19) Bergmann, K. (1975). The epidemiology of senile dementia. In Contemporary Psychiatry, British Journal of Psychiatry Special Publication No.9, ed., Silverstone, T., and Barraclough, B. Ashford, Kent: Headley Brothers Ltd., p. 100.

- 20) Nielsen, J. (1968). Epidemiology of senile dementia. In *Senile Dementia*, ed., Muller, C., and Ciompi, L. Bern: Hans Huber Publishers, p. 51.
- 21) Bergmann, K. (1977). Chronic brain failure -epidemiological aspects. *Age and Ageing*, 6, Suppl., p.4.
- 22) Slater, E., and Roth, M. (1969). In *Clinical Psychiatry*, (3rd ed.). London: Bailliere, Tindall and Cassell, p. 607.
- 23) Lishman, W.A. (1978). In *Organic Psychiatry*, (1st ed). Oxford, London, Edinburgh: Blackwell Scientific Publications, p. 528.
- 24) Sourander, P. (1970). In *Alzheimer's Disease and Related Conditions*, ed., Wolstenholme, G.E.W., and O'Connor, M. London: J. and A. Churchill, p. 70.
- 25) Corsellis, J.A.N. (1976). Ageing and the Dementias. In *Greenfield's Neuropathology* (3rd ed.), ed. Blackwood, W., and Corsellis, J.A.N. London: Edward Arnold, p. 796.
- 26) Dayan, A.D. (1978). The central nervous system a. Neuropathology of ageing. In *Textbook of Geriatric Medicine and Gerontology* (2nd ed.), ed. Brocklehurst, J.C. Edinburgh, London and New York: Churchill Livingstone, p. 158.

- 27) Scheibel, A.B. (1978). Structural aspects of the ageing brain: Spine systems and the dendritic arbor. In Alzheimer's Disease: Senile Dementia and Related Disorders, ed. Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 353.
- 28) Friede, R.L. and Magee, K.R. (1962). Alzheimer's disease. Presentation of a case with pathologic and enzymatic-histochemical observations. *Neurology*, 12, 213.
- 29) Terry, R.D. (1963). The fine structure of neurofibrillary tangles in Alzheimer's disease. *Journal of Neuropathology and Experimental Neurology*, 22, 629.
- 30) Terry, R.D., Gonatas, N.K. and Weiss, M. (1964). Ultrastructural studies in Alzheimer's presenile dementia. *American Journal of Pathology*. 44, 269.
- 31) Luse, S.A., and Smith, K.R. (1964). The ultrastructure of senile plaques. *American Journal of Pathology*, 44, 553.
- 32) Kidd, M. (1964). Alzheimer's disease - an electron microscopical study. *Brain*, 87, 307.
- 33) Krigman, M.R., Feldman, R.G., and Bensch, K. (1965). Alzheimer's presenile dementia. A histochemical and electron microscopic study. *Laboratory Investigation*, 14, 381.

- 34) Terry, R.D., and Wisniewski, H. (1970). The ultrastructure of the neurofibrillary tangle and the senile plaque. In *Alzheimer's Disease and Related Conditions*, ed. Wolstenholme, G.E.W., and O'Connor, M. London: J and A. Churchill, p. 145.
- 35) Terry, R.D. (1978). Ultrastructural alterations in senile dementia. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed. Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 375.
- 36) Wisniewski, H.M. (1979). Neurofibrillary and synaptic pathology in senile dementia of the Alzheimer type (SDAT). In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits*, ed. Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 7.
- 37) Wisniewski, H.M., Narang, H.K., and Terry, R.D. (1976). Neurofibrillary tangles of paired helical filaments. *Journal of the Neurological Sciences*, 27, 173.
- 38) Iqbal, K., Grundke-Iqbal, I., Wisniewski, H.M., and Terry, R.D. (1978). Neurofibers in Alzheimer dementia and other conditions. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed. Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 409.
- 39) Mandybur, T.I. (1975). The incidence of cerebral

amyloid angiopathy in Alzheimer's disease. *Neurology*, 25, 120.

40) Smith, C.M., and Swash, M. (1979). Possible biochemical basis of memory disorder in Alzheimer's disease. *Age and Ageing*, 8, 289.

41) Drachman, D.A., and Leavitt, J. (1974). Human memory and the cholinergic system. A relationship to ageing? *Archives of Neurology*, 30, 113.

42) Sitaram, N., and Weingartner, H. (1979). Cholinergic mechanisms in human memory. In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits*, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 159.

43) Davies, P., and Maloney, A.J.F. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet*, ii, 1403.

44) Perry, E.K., Perry, R.H., Blessed, G., and Tomlinson, B.E. (1977). Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet*, i, 189.

45) White, P., Hiley, C.R., Goodhardt, M.J., Carrasco, L.H., Keet, J.P., Williams, I.E.I., and Bowen, D.M. (1977). Neocortical cholinergic neurons in elderly people. *Lancet*, i, 668.

- 46) Spillane, J.A., White, P., Goodhardt, M.J., Flack, R.H.A., Bowen, D.M., and Davison, A.N. (1977). Selective vulnerability of neurones in organic dementia. *Nature*, 266, 558.
- 47) Boyd, W.D., Graham-White, J., Blackwood, G., Glen, I., and McQueen, J. (1977). Clinical effects of choline in Alzheimer senile dementia. *Lancet*, ii, 711.
- 48) Etienne, P., Gauthier, S., Johnson, G., Collier, B., Mendis, T., Dastoor, D., Cole, M., and Muller, H.F. (1978). Clinical effects of choline in Alzheimer's disease. *Lancet*, i, 508.
- 49) Smith, C.M., Swash, M., Exton-Smith, A.N., Phillips, M.J., Overstall, P.W., Piper, M.E., and Bailey, M.R. (1978). Choline therapy in Alzheimer's disease. *Lancet*, ii, 318.
- 50) Signoret, J.L., Whiteley, A., and Lhermitte, F. (1978). Influence of choline on amnesia in early Alzheimer's disease. *Lancet*, ii, 837.
- 51) Renvoize, E.B., and Jerram, T. (1979). Choline in Alzheimer's disease. *New England Journal of Medicine*, 301, 330.
- 52) Christie, J.E., Glen, A.I.M., Yates, C.M., Blackburn, I.M., Shering, A., Jellinek, E.H., and Zeisel, S. (1979).

Choline and lecithin effects on CSF choline levels and on cognitive function in Alzheimer pre-senile dementia. In Alzheimer's Disease. Early recognition of Potentially Reversible Deficits, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 163.

53) Etienne, P., Gauthier, S., Dastoor, D., Collier, B., and Ratner, J. (1978). Lecithin in Alzheimer's disease. Lancet, ii, 1206.

54) Corser, C.M., Baikie, E., and Brown, E. (1979). Effect of lecithin in senile dementia: a report of four cases. In Alzheimer's Disease. Early recognition of Potentially Reversible Deficits, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 169.

55) Peters, B.H., and Levin, H.S. (1979). Effects of physostigmine and lecithin on memory in Alzheimer's disease. Annals of Neurology, 6, 219.

56) Smith, C.M., and Swash, M. (1979). Physostigmine in Alzheimer's disease. Lancet, i, 42.

57) Anon. (1980). Alzheimer's disease. British Medical Journal, 281, 1374.

58) Hirano, A., and Zimmerman, H.M. (1962). Alzheimer's neurofibrillary changes. A topographic study. Archives of Neurology, 7, 227.

- 59) Wisniewski, K., Jervis, G.A., Moretz, R.C., and Wisniewski, H.M. (1979). Alzheimer neurofibrillary tangles in diseases other than senile and presenile dementia. *Annals of Neurology*, 5, 288.
- 60) Olson, M.I., and Shaw, C. (1969). Presenile dementia and Alzheimer's disease in mongolism. *Brain*, 92, 147.
- 61) Ellis, W.G., McCulloch, J.R., and Corley, C.L. (1974). Presenile dementia in Down's syndrome. Ultrastructural identity with Alzheimer's disease. *Neurology*, 24, 101.
- 62) Whalley, L.J., and Buckton, K.E. (1979). Genetic factors in Alzheimer's disease. In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits*, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 36.
- 63) Nielsen, J. (1968). Chromosomes in senile dementia. In *Senile Dementia*, ed., Muller, C., and Ciompi, L. Bern: Hans Huber Publishers, p. 59.
- 64) Nielsen, J. (1970). Chromosomes in senile, presenile, and arteriosclerotic dementia. *Journal of Gerontology*, 25, 312.
- 65) Mark, J., and Brun, A. (1973). Chromosomal deviations in Alzheimer's disease compared to those in senescence and

senile dementia. Geront. Clin., 15, 253.

66) Jarvik, L.F., Yen, F., and Moralishvili, E. (1974). Chromosome examinations in aging institutionalized women. Journal of Gerontology, 29, 269.

67) Jarvik, L.F., Yen, F., and Goldstein, F. (1974). Chromosomes and mental status. Archives of General Psychiatry, 30, 186.

68) Brun, A., Gustafson, L., and Mitelman, F. (1978). Normal chromosome banding pattern in Alzheimer's disease. Gerontology, 24, 369.

69) Cook, R.H., Ward, B.E., and Austin, J.H. (1979). Studies in Aging of the brain: IV. Familial Alzheimer disease: Relation to transmissible dementia, aneuploidy, and microtubular defects. Neurology, 29, 1402.

70) Nordenson, I., Adolfsson, R., Beckman, G., Bucht, G., and Winblad, B. (1980). Chromosomal abnormality in dementia of Alzheimer type. Lancet, i, 481.

71) Galloway, S.M., and Buckton, K.E. (1978). Aneuploidy and ageing: Chromosome studies on a random sample of the population using G banding. Cytogenetics and Cell Genetics, 20, 78.

- 73) Klatzo, I., Wisniewski, H., and Streicher, E. (1965). Experimental production of neurofibrillary degeneration. I. Light microscopic observations. *Journal of Neuropathology and Experimental Neurology*, 24, 187.
- 74) Crapper, D.R., Karlik, S., and De Boni, U. (1978). Aluminium and other metals in senile (Alzheimer) dementia. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 471.
- 75) Crapper, D.R., Krishnan, S.S., and Quittkat, S. (1976). Aluminium neurofibrillary degeneration and Alzheimer's disease. *Brain*, 99, 67.
- 76) McDermott, J.R., Smith, A.I., Ward, M.K., Parkinson, I.S., and Kerr, D.N.S. (1978). Brain-aluminium concentration in dialysis encephalopathy. *Lancet*, i, 901.
- 77) Elliott, H.L., Dryburgh, F., Fell, G.S., Sabet, S., and Macdougall, A.I. (1978). Aluminium toxicity during regular haemodialysis. *British Medical Journal*, i, 1101.
- 78) McDermott, J.R., Smith, A.I., Iqbal, K., and Wisniewski, H.M. (1977). Aluminium and Alzheimer's disease. *Lancet*, ii, 710.
- 79) McDermott, J.R., Smith, A.I., Iqbal, K., and Wisniewski,

H.M. (1979). Brain aluminium in aging and Alzheimer disease. *Neurology*, 29, 809.

80) Delaney, J.F. (1979). Spinal fluid aluminium levels in patients with Alzheimer disease. *Annals of Neurology*, 5, 580.

81) Kral, V.A. (1962). Senescent forgetfulness: Benign and malignant. *The Canadian Medical Association Journal*, 86, 257.

82) Kral, V.A. (1978). Benign senescent forgetfulness. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 47.

83) Blessed, G., Tomlinson, B.E., and Roth, M. (1968). The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *British Journal of Psychiatry*, 114, 797.

84) Roth, M. (1971). Classification and aetiology in mental disorders of old age: Some recent developments. In *Recent Developments in Psychogeriatrics*, ed., Kay, D.W.K., and Walk, A. *British Journal of Psychiatry Special Publication No 6*. Ashford, Kent: Headley Brothers Ltd., p.1.

85) Dayan, A.D. (1970). Quantitative histological studies

on the aged human brain. I. Senile plaques and neurofibrillary tangles in "normal" patients. *Acta Neuropathologica*, 16, 85.

86) Dayan, A.D. (1970). Quantitative histological studies on the aged human brain. II. Senile plaques and neurofibrillary tangles in senile dementia. *Acta Neuropathologica*, 16, 95.

87) Tomlinson, B.E., Blessed, G., and Roth, M. (1970). Observations on the brains of demented old people. *Journal of the Neurological Sciences*, 11, 205.

88) Bowen, D.M. (1979). Neurochemical findings in Alzheimer's disease. In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits*, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 17.

89) Bowen, D.M., Spillane, J.A., Curzon, G., Meier-Ruge, W., White, P., Goodhardt, M.J., Iwangoff, P., and Davison, A.N. (1979). Accelerated ageing or selective neuronal loss as an important cause of dementia? *Lancet*, i, 11.

90) Perry, E.K., Perry, R.H., Gibson, P.H., Blessed, G., and Tomlinson, B.E. (1977). A cholinergic connection between normal aging and senile dementia in the human hippocampus. *Neuroscience Letters*, 6, 85.

- 91) Perry, E.K., Tomlinson, B.E., Blessed, G., Bergmann, K., Gibson, P.H., and Perry, R.H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal*, 2, 1457.
- 92) Perry, E.K. (1979). Correlations between psychiatric, neuropathological and biochemical findings in Alzheimer's disease. In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits.* ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 27.
- 93) Perry, E.K. (1980). The cholinergic system in old age and Alzheimer's disease. *Age and Ageing*, 9, 1.
- 94) Pratt, R.T.C. (1967). Senile dementia and the pre-senile dementias. In *The Genetics of Neurological Disorders.* London: Oxford University Press, p. 71.
- 95) Pratt, R.T.C. (1970). The genetics of Alzheimer's disease. In *Alzheimer's Disease and Related Conditions*, ed., Wolstenholme, G.E.W., and O'Connor, M. London: Churchill, p. 137.
- 96) Slater, E., and Cowie, V. (1971). Senescence, senile and pre-senile dementias. In *The Genetics of Mental Disorders.* London: Oxford University Press, p. 122.

- 97) Jarvik, L.F. (1978). Genetic factors and chromosomal aberrations in Alzheimer's disease, senile dementia, and related disorders. In Alzheimer's Disease: Senile Dementia and Related Disorders, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 273.
- 98) Feldman, R.G., Chandler, K.A., Levy, L.L., and Glaser, G.H. (1963). Familial Alzheimer's disease. *Neurology*, 13, 811.
- 99) Wheelan, L. (1959). Familial Alzheimer's disease. *Annals of Human Genetics*, 23, 300.
- 100) Heston, L.L., Lowther, D.L.W., and Leventhal, C.M. (1966). Alzheimer's disease. A family study. *Archives of Neurology*, 15, 225.
- 101) Landy, P.J., and Bain, B.J. (1970). Alzheimer's disease in siblings. *The Medical Journal of Australia*, 2, 832.
- 102) Heston, L.L., and Mastri, A.R. (1977). The genetics of Alzheimer's disease: Associations with haematologic malignancy and Down's syndrome. *Archives of General Psychiatry*, 34, 976.
- 103) Heston, L.L. (1977). Alzheimer's disease, trisomy 21, and myeloproliferative disorders: Associations suggesting a genetic diathesis. *Science*, 196, 322.

- 104) Clarke, C.A. (1978). Chromosomes and leukaemia. In *Progress in Clinical Medicine*, ed., Horler, A.R., and Foster, J.B. Edinburgh: Churchill Livingstone, p. 24.
- 105) Larsson, T., Sjogren, T., and Jacobson, G. (1963). Senile dementia. A clinical, sociomedical and genetic study. *Acta Psychiatrica Scandinavica*, 39, Suppl. 167.
- 106) Kallmann, F.J. (1956). Genetic aspects of mental disorders in later life. In *Mental Disorders in Later Life*, ed., Kaplan, O.J. London: Oxford University Press, p. 26.
- 107) Walford, R.L. (1969). *The Immunologic Theory of Aging*. Copenhagen: Munksgaard.
- 108) Mackay, I.R. (1972). Ageing and immunological function in man. *Gerontologia*, 18, 285.
- 109) Greenberg, L.J., and Yunis, E.J. (1972). Immunologic control of aging: A possible primary event. *Gerontologia*, 18, 247.
- 110) Bellamy, D. (1973). The thymus in relation to problems of cellular growth and ageing. *Gerontologia*, 19, 162.
- 111) Adler, W.H., Jones, K.H., and Nariuchi, H. (1977).

Ageing and immune function. In *Recent Advances in Clinical Immunology*, ed., Thompson, R.A. Edinburgh, London and New York: Churchill Livingstone, p. 77.

112) Kelsall, M.A. (1966). Aging on mast cells and plasmacytes in the brain of hamsters. *The Anatomical Record*, 154, 727.

113) Blumenthal, H.T. (1976). Immunological aspects of the aging brain. In *Neurobiology of Aging*, ed., Terry, R.D., and Gershon, S. New York: Raven Press, p. 313.

114) Rowley, M.J., Buchanan, H., and Mackay, I.R. (1968). Reciprocal change with age in antibody to extrinsic and intrinsic antigens. *Lancet*, ii, 24.

115) Heidrick, M.L., and Makinodan, T. (1972). Nature of cellular deficiencies in age-related decline of the immune system. *Gerontologia*, 18, 305.

116) Makinodan, T., Albright, J.W., Good, P.I., Peter, C.P., and Heidrick, M.L. (1976). Reduced humoral immune activity in long-lived old mice: An approach to elucidating its mechanisms. *Immunology*, 31, 903.

117) Hallgren, H.M., Buckley, C.E., Gilbertsen, V.A., and Yunis, E.J. (1973). Lymphocyte phytohemagglutinin responsiveness, immunoglobulins and autoantibodies in aging

humans. *Journal of Immunology*, 111, 1101.

118) Cassidy, J.T., Nordby, G.L., and Dodge, H.J. (1974).
Biologic variation of human serum immunoglobulin
concentrations: Sex-age specific effects. *Journal of
Chronic Diseases*, 27, 507.

119) Radl, J., Sepers, J.M., Skvaril, F., Morell, A., and
Hijmans, W. (1975). Immunoglobulin patterns in humans
over 95 years of age. *Clinical and Experimental Immunology*,
22, 84.

120) Hallen, J. (1963). Frequency of "abnormal" serum
globulins (M-components) in the aged. *Acta Medica
Scandinavica*, 173, 737.

121) Axelsson, U., Bachmann, R., and Hallen, J. (1966).
Frequency of pathological proteins (M-components) in 6,995
sera from an adult population. *Acta Medica Scandinavica*,
179, 235.

122) Englisova, M., Englis, M., Kyrál, V., Kourilek, K.,
and Dvorak, K. (1968). Changes of immunoglobulin
synthesis in old people. *Experimental Gerontology*, 3, 125.

123) Augener, W., Cohnen, G., Reuter, A., and Brittinger, G.
(1974). Decrease of T lymphocytes during ageing. *Lancet*, i,
1164.

- 124) Diaz-Jouanen, E., Strickland, R.G., and Williams, R.C. (1975). Studies of human lymphocytes in the newborn and the aged. *American Journal of Medicine*, 58, 620.
- 125) Girard, J.P., Paychere, M., Cuevas, M., and Fernandes, B. (1977). Cell-mediated immunity in an ageing population. *Clinical and Experimental Immunology*, 27, 85.
- 126) Clot, J., Charmasson, E., Brochier, J. (1978). Age-dependent changes of human blood lymphocyte subpopulations. *Clinical and Experimental Immunology*, 32, 346.
- 127) Reddy, M.M., and Goh, K. (1979). B and T lymphocytes in man iv. Circulating B, T and "Null" lymphocytes in aging population. *Journal of Gerontology*, 34, 5.
- 128) Giannini, D., and Sloan, R.S. (1957). A tuberculin survey of 1285 adults with special reference to the elderly. *Lancet*, i, 525.
- 129) Waldorf, D.S., Willkens, R.F., and Decker, J.L. (1968). Impaired delayed hypersensitivity in an aging population. *Journal of the American Medical Association*, 203, 831.
- 130) Roberts-Thomson, I.C., Whittingham, S., Youngchaiyud, U., and Mackay, I.R. (1974). Ageing, immune response, and mortality. *Lancet*, ii, 368.

131) Carosella, E.D., Mochanko, K., and Braun, M. (1974). Rosette-forming T cells in human peripheral blood at different ages. *Cellular Immunology*, 12, 323.

132) Smith, M.A., Evans, J., and Steel, C.M. (1974). Age-related variation in proportion of circulating T cells. *Lancet*, ii, 922.

133) Pisciotta, A.V., Westring, D.W., De Prey, C., and Walsh, B. (1967). Mitogenic effect of phytohaemagglutinin at different ages. *Nature*, 215, 193.

134) Ippoliti, G., Marini, G., Casirola, G., and Invernizzi, R. (1974). T lymphocytes and immunoglobulins in the aged. *Lancet*, ii, 953.

135) Geraint James, D. (1979). General introduction. In *Clinical Neuroimmunology*, ed., Clifford Rose, F. London: Blackwell Scientific Publications, p.3.

136) Kay, M.M.B. (1978). Effect of age on T cell differentiation. *Federation Proceedings Federation of American Societies for Experimental Biology*, 37, 1241.

137) Hirokawa, K., and Makinodan, T. (1975). Thymic involution: Effect on T cell differentiation. *Journal of Immunology*, 114, 1659.

- 138) Hackett, E., Beech, M, and Forbes, I.J. (1960). Thyroglobulin antibodies in patients without clinical disease of the thyroid gland. *Lancet*, ii, 402.
- 139) Heimer, R., Levin, F.M., and Rudd, E. (1963). Globulins resembling rheumatoid factor in serum of the aged. *American Journal of Medicine*, 35, 175.
- 140) Cammarata, R.J., Rodnan, G.P., and Fennell, R.H. (1967). Serum anti- γ - globulin and antinuclear factors in the aged. *Journal of the American Medical Association*, 199, 455.
- 141) Hooper, B., Whittingham, S., Matthews, J.D., Mackay, I.R., and Curnow, D.H. (1972). Autoimmunity in a rural community. *Clinical and Experimental Immunology*, 12, 79.
- 142) Valtuena, J.P., Guilabert, M.I.G., Perez, M.A., Crespo, F., and Alonso, R.V. (1973). Autoimmunity, immunoglobulins and T lymphocyte population in old age. *Biomedicine*, 19, 301.
- 143) Nandy, K. (1972). Brain reactive antibodies in mouse serum as a function of age. *Journal of Gerontology*, 27, 173.
- 144) Nandy, K. (1977). Immune reactions in aging brain and senile dementia. In *the Aging Brain and Senile Dementia*,

ed., Nandy, K., and Sherwin, I. New York: Plenum Press, p. 181.

145) Nandy, K. (1978). Brain-reactive antibodies in aging and senile dementia. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 503.

146) Ingram, C.R., Phegan, K.J., and Blumenthal, H.T. (1974). Significance of an aging-linked neuron binding gamma globulin fraction of human sera. *Journal of Gerontology*, 29, 20.

147) Field, E.J., and Shenton, B.K. (1973). Emergence of new antigens in ageing tissues. *Gerontologia*, 19, 211.

148) Krakauer, R.S., Waldmann, T.A., and Strober, W. (1976). Loss of suppressor T cells in adult NZB/NZW mice. *Journal of Experimental Medicine*, 144, 662.

149) Hallgren, H.M., and Yunis, E.J. (1977). Suppressor lymphocytes in young and aged humans. *Journal of Immunology*, 118, 2004.

150) Kishimoto, S., Tomino, S., Mitsuya, H., and Fujiwara, H., (1979). Age-related changes in suppressor functions of human T cells. *Journal of Immunology*, 123, 1586.

- 151) Thung, P.J. (1957). The relation between amyloid and ageing in comparative pathology. *Gerontologia*, 1, 234.
- 152) Thung, P.J., (1957). Senile amyloidosis in mice. *Gerontologia*, 1, 259.
- 153) Schwartz, P. (1970). Amyloidosis. Springfield, Illinois: Charles C. Thomas.
- 154) Schwartz, P. (1972). Amyloid degeneration and tuberculosis in the aged. *Gerontologia*, 18, 321.
- 155) Anon. (1981). The senile amyloidoses. *British Medical Journal*, 282, 846.
- 156) Lee, H.Y., and Kaufmann, W. (1957). Cardiac amyloidosis in the aged. *Archives of Pathology*, 64, 494.
- 157) Buerger, L., and Braunstein, H. (1960). Senile cardiac amyloidosis. *American Journal of Medicine*, 28, 357.
- 158) de Jager, H., and Stam, F.C. (1962). Pathology and histochemistry of amyloidosis in old age. *Gerontologia*, 6, 19.
- 159) Pomerance, A. (1965). Senile cardiac amyloidosis. *British Heart Journal*, 27, 711.

160) Hodkinson, H.M., and Pomerance, A. (1977). The clinical significance of senile cardiac amyloidosis: A prospective clinico-pathological study. *Quarterly Journal of Medicine*, 183, 381.

161) Hodkinson, M. (1979). Senile cardiac amyloidosis: No longer just a curiosity. *Geriatric Medicine*, September, p. 81.

162) Berns, A.W., Owens, C.T., and Blumenthal, H.T. (1964). A histo- and immunopathologic study of the vessels and islets of langerhans of the pancreas in diabetes mellitus. *Journal of Gerontology*, 19, 179.

163) Schwartz, P., Kurucz, J., and Kurucz, A. (1964). Recent observations on senile cerebral changes and their pathogenesis. *Journal of the American Geriatrics Society*, 12, 908.

164) Schwartz, P., (1965). Senile cerebral, pancreatic insular and cardiac amyloidosis. *Transactions of the New York Academy of Sciences*, 27, 393.

165) Franklin, E.C., Rosenthal, C.J., Pras, M., and Levin, M. (1976). Recent progress in amyloid. In *the Role of Immunological Factors in Infectious, Allergic, and Auto-immune Processes*, ed., Beers, Jr., R.F., and Bassett, E.G. New York: Raven Press, p. 163.

- 166) Ignaczak, T.F., Sipe, J.D., Linke, R.P., and Glenner, G.G. (1977). Immunochemical studies on the nature of the serum component (SAA) related to secondary amyloidosis. *Journal of Laboratory and Clinical Medicine*, 89, 1092.
- 167) Benson, M.D., Skinner, M., Lian, J., Cohen, A.S., and Shirahama, T. (1974). Amyloid serum component: Relationship to aging. *Federation Proceedings*, 33, 618.
- 168) Westermark, P., (1975). Amyloid of human islets of langerhans. *Acta Pathologica et Microbiologica Scandinavica*, 83, 439.
- 169) Westermark, P., Natvig, J.B., and Johansson, B., (1977). Characterization of an amyloid fibril protein from senile cardiac amyloid. *Journal of Experimental Medicine*, 146, 631.
- 170) Cornwell, G.G., Natvig, J.B., Westermark, P., and Husby, G. (1978). Senile cardiac amyloid: Demonstration of a unique fibril protein in tissue sections. *Journal of Immunology*, 120, 1385.
- 171) Burnet, F.M. (1970). An immunological approach to ageing. *Lancet*, ii, 358.
- 172) Walford, R.L. (1970). Antibody diversity, histocompatibility systems, disease states, and ageing. *Lancet*, ii, 1226.

173) Hughes, R.A.C., and Leibowitz, S. (1977). Neurological disease. In *Immunology in Medicine*, ed., Holborow, E.J., and Reeves, W.G. London: Academic Press, p. 869.

174) Link, H. (1979). Some aspects of immune reactions of the brain. In *Clinical Neuroimmunology*, ed., Rose, F.C. London: Blackwell Scientific Publications, p. 12.

175) Rapoport, S.I. (1977). Reversible modification of blood-brain barrier permeability to proteins. In *The Aging Brain and Senile Dementia*, ed., Nandy, K., and Sherwin, I. New York: Plenum Press, p. 197.

176) Behan, P.O., and Currie, S. (1978). Immune function and the nervous system. In *Clinical Neuroimmunology* London, Philadelphia, Toronto: W.B. Saunders Company Ltd., p. 18.

177) Whittingham, S., Lennon, V., Mackay, I.R., Davies, G. V., and Davies, B. (1970). Absence of brain antibodies in senile dementia. *British Journal of Psychiatry*, 116, 447.

178) Mayer, P.P., Chughtai, M.A., and Cape, R.D.T. (1976). An immunological approach to dementia in the elderly. *Age and Ageing*, 5, 164.

179) Tkach, J.R., and Hokama, Y. (1970). Autoimmunity in chronic brain syndrome. *Archives of General Psychiatry*, 23, 61.

- 180) Behan, P.O., and Feldman, R.G., (1970). Serum proteins, amyloid and Alzheimer's disease. *Journal of the American Geriatrics Society*, 18, 792.
- 181) Kalter, S., and Kelly, S. (1975). Alzheimer's disease. Evaluation of immunologic indices. *New York State Journal of Medicine*, July, p. 1222.
- 182) Eisdorfer, C., Cohen, D., and Buckley, C.E. (1978). Serum immunoglobulins and cognition in the impaired elderly. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 401.
- 183) Cohen, D., and Eisdorfer, C. (1980). Serum immunoglobulins and cognitive status in the elderly: 1. A population study. *British Journal of Psychiatry*, 136, 33.
- 184) Eisdorfer, C., and Cohen, D. (1980). Serum immunoglobulins and cognitive status in the elderly: 2. An immunological-behavioral relationship? *British Journal of Psychiatry*, 136, 40.
- 185) Tavalato, B., and Argentiero, V. (1980). Immunological indices in presenile Alzheimer's disease. *Journal of the Neurological Sciences*, 46, 325.
- 186) Williams, A., Papadopoulos, N., and Chase, T.N. (1980).

Demonstration of CSF gamma-globulin banding in presenile dementia. *Neurology*, 30, 882.

187) Behan, P.O., and Behan, W.M.H. (1979). Possible immunological factors in Alzheimer's disease. In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits*, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 33.

188) Mehne, P., et al. (1976). A serogenetic approach to the study of Alzheimer's disease. *Aktuelle Gerontologie*, 6, 259. (Translation).

189) Mathews, W.H. (1954). Primary systemic amyloidosis. *The American Journal of the Medical Sciences*, 228, 317.

190) Cohen, A.S. (1967). Amyloidosis. *New England Journal of Medicine*, 277, 522.

191) Cohen, A.S. (1967). Amyloidosis. *New England Journal of Medicine*, 277, 574.

192) Cohen, A.S. (1967). Amyloidosis. *New England Journal of Medicine*, 277, 628.

193) Hobbs, J.R. (1973). An A B C of amyloid. *Proceedings of the Royal Society of Medicine*, 66, 705.

- 194) Glenner, G.G., Terry, W.D., and Iversky, C. (1973). Amyloidosis: Its nature and pathogenesis. *Seminars in Haematology*, 10, 65.
- 195) Rosenthal, C.J., and Franklin, E.C. (1977). Amyloidosis and amyloid proteins. In *Recent Advances in Clinical Immunology*, ed., Thompson, R.A. Edinburgh, London and New York: Churchill Livingstone, p. 41.
- 196) Pruzanski, W. (1977). Amyloidogenesis - Theories and facts. *Journal of Rheumatology*, 4, 219.
- 197) Glenner, G.G. (1980). Amyloid deposits and amyloidosis. *New England Journal of Medicine*, 302, 1283.
- 198) Glenner, G.G. (1980). Amyloid deposits and amyloidosis. *New England Journal of Medicine*, 302, 1333.
- 199) Isobe, T., and Osserman, E.F. (1974). Patterns of amyloidosis and their association with plasma-cell dyscrasia, monoclonal immunoglobulins and Bence-Jones proteins. *New England Journal of Medicine*, 290, 473.
- 200) Haberland, C. (1964). Primary systemic amyloidosis. Cerebral involvement and senile plaque formation. *Journal of Neuropathology and Experimental Neurology*, 23, 135.
- 201) De Navasquez, S., and Treble, H.A. (1938). A case of

primary generalized amyloid disease with involvement of the nerves. *Brain*, 61, 116.

202) Andersson, R. (1970). Hereditary amyloidosis with polyneuropathy. *Acta Medica Scandinavica*, 188, 85.

203) Andersson, R., and Blom, S. (1972). Neurophysiological studies in hereditary amyloidosis with polyneuropathy. *Acta Medica Scandinavica*, 191, 233.

204) Kelly, Jr., J.J., Kyle, R.A., O'Brien, P.C., and Dyck, P.J. (1979). The natural history of peripheral neuropathy in primary systemic amyloidosis. *Annals of Neurology*, 6, 1.

205) Skinner, M., Cohen, A.S., Shirahama, T., and Cathcart, E.S. (1974). P-component (pentagonal unit) of amyloid: Isolation, characterization, and sequence analysis. *Journal of Laboratory and Clinical Medicine*, 84, 604.

206) Glenner, G.G., Ein, D., Eanes, E.D., Bladen, H.A., Terry, W., and Page, D.L. (1971). Creation of "amyloid" fibrils from Bence Jones proteins in vitro. *Science*, 174, 712.

207) Glenner, G.G., Ein, D., and Terry, W.D. (1972). The immunoglobulin origin of amyloid. *American Journal of Medicine*, 52, 141.

- 208) Anon. (1973). Immunoglobulin origin of amyloid. Lancet, i, 1046.
- 209) Benditt, E.P., and Eriksen, N. (1971). Chemical classes of amyloid substance. American Journal of Pathology, 65, 231.
- 210) Levin, M., Franklin, E.C., Frangione, B., and Pras, M. (1972). The amino acid sequence of a major nonimmunoglobulin component of some amyloid fibrils. The Journal of Clinical Investigation, 51, 2773.
- 211) Husby, G., and Natvig, J.B. (1974). A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. The Journal of Clinical Investigation, 53, 1054.
- 212) Meretoja, J., Natvig, J.B., and Husby, G. (1976). Amyloid-related serum protein (SAA) in patients with inherited amyloidosis and certain viral conditions. Scandanavian Journal of Immunology, 5, 169.
- 213) Scheinberg, M.A., and Cathcart, E.S. (1978). New concepts in the pathogenesis of primary and secondary amyloid disease. Clinical and Experimental Immunology, 33, 185.
- 214) Glenner, G.G., Eanes, E.D., Bladen, H.A., Linke, R.P., and Termine, J.D. (1974). β -pleated sheet fibrils. A

- comparison of native amyloid with synthetic protein fibrils. *The Journal of Histochemistry and Cytochemistry*, 22, 1141.
- 215) Westermark, P., Natvig, J.B., Anders, R.F., Sletten, K., and Husby, G. (1976). Coexistence of protein AA and immunoglobulin light - chain fragments in amyloid fibrils. *Scandinavian Journal of Immunology*, 5, 31.
- 216) Glenner, G.G. (1978). Current knowledge of amyloid deposits as applied to senile plaques and congophilic angiopathy. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 493.
- 217) Glenner, G.G. (1979). Congophilic microangiopathy in the pathogenesis of Alzheimer's syndrome (presenile dementia). *Medical Hypotheses*, 5, 1231.
- 218) Bruni, J., Bilbao, J.M., and Pritzker, K.P.H. (1977). Vascular amyloid in the aging central nervous system. Clinico-pathological study and literature review. *The Canadian Journal of Neurological Sciences*, 4, 239.
- 219) Horst, van der, L., Stam, F.C., and Wigboldus, J.M. (1960). Amyloidosis in senile and pre-senile involutional processes of the central nervous system. *The Journal of Nervous and Mental Disease*, 130, 578.

220) Immunology Today, 2, 10, October 1981. Centre page diagram.

221) Powers, J.M., and Spicer, S.S. (1977). Histochemical similarity of senile plaque amyloid to apudamyloid. Virchows Arch. A Path. Anat. and Histol., 376, 107.

222) Bartoli, M., Antonutto, G., and Bianchi, C. (1976). Plaques séniles et immunoamyloïde. Arch. Anat. Cytol. Path., 24, 287.

223) Ishii, T., Haga, S., and Shimizu, F. (1975). Identification of components of immunoglobulins in senile plaques by means of fluorescent antibody technique. Acta Neuropathologica, 32, 157.

224) Ishii, T., and Haga, S. (1976). Immuno-electron microscopic localization of immunoglobulins in amyloid fibrils of senile plaques. Acta Neuropathologica, 36, 243.

225) Miyakawa, T., Sumiyoshi, S., Murayama, E., and Deshimaru, M. (1974). Ultrastructure of capillary plaque-like degeneration in senile dementia. Mechanism of amyloid production. Acta Neuropathologica, 29, 229.

226) Miyakawa, T., and Vehara, Y. (1979). Observations of amyloid angiopathy and senile plaques by the scanning

electron microscope. *Acta Neuropathologica*, 48, 153.

227) Horta-Barbosa, L., Fuccillo, D.A., and Sever, J.L.

(1971). Chronic viral infections of the central nervous system. *Journal of the American Medical Association*, 218, 1185.

228) Pattison, I.H. (1972). Scrapie - A personal view.

Journal of Clinical Pathology, 25, Suppl. (Roy. Coll. Path.), 6, 110.

229) Hadlow, W.J. (1972). Animal models of slow viral

disease. In *Membranes and Viruses in Immunopathology*, ed., Day, S.B., and Good, R.A. New York: Academic Press, p. 381.

230) Hunter, G.D., Kimberlin, R.H., Collis, S., and

Millson, G.C., (1973). Viral and non-viral properties of the scrapie agent. *Annals of Clinical Research*, 5, 262.

231) Gajdusek, D.C. (1978). Unconventional viruses. In

Human Diseases Caused by Viruses, ed., Rothschild, H., Allison, Jr., F., and Howe, C. New York: Oxford University Press, p. 233.

232) Gajdusek, D.C. (1977). Unconventional viruses and the origin and disappearance of kuru. *Science*, 197, 943.

- 233) Gajdusek, D.C. (1972). Spongiform virus encephalopathies. *Journal of Clinical Pathology*, 25, Suppl. (Roy. Coll. Path), 6, 78.
- 234) Marx, J.L. (1973). Slow viruses (II): The unconventional agents. *Science*, 181, 44.
- 235) Gajdusek, D.C. (1973). Kuru and Creutzfeldt-Jakob disease. *Annals of Clinical Research*, 5, 254.
- 236) Webb, H.E. (1977). Slow virus diseases of the central nervous system. In *Recent Advances in Medicine*, Number 17, ed., Baron, D.N., Compston, N, and Dawson, A.M. Edinburgh, London and New York: Churchill Livingstone, p. 83.
- 237) Brody, J.A., and Gibbs, Jr., C.J. (1976). Chronic neurological diseases. In *Viral Infections of Humans*, ed., Evans, A.S. New York: John Wiley and Sons, P. 519.
- 238) Adams, D.H., and Bell, T.M. (1976). *Slow Viruses*, Massachusetts: Addison-Wesley Publishing Company.
- 239) Gibbs, Jr., C.J., and Gajdusek, D.C. (1978). Subacute spongiform virus encephalopathies: The transmissible virus dementias. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 559.

- 240) Adams, J.M. (1979) Immunology of slow infections. In Immunological Aspects of Infectious Diseases, ed., Dick, G. Lancaster, England: MTP Press Limited, p. 497.
- 241) Johnson, R.T. (1970). Subacute sclerosing panencephalitis. *Journal of Infectious Diseases*, 121, 227.
- 242) Connolly, J.H. (1972). Subacute sclerosing panencephalitis. *Journal of Clinical Pathology*, 25, Suppl. (Roy. Coll. Path.), 6, 73.
- 243) Dawson, J.R. (1933). Cellular inclusions in cerebral lesions of lethargic encephalitis. *American Journal of Pathology*, 9, 7.
- 244) Dawson, J.R. (1934). Cellular inclusions in cerebral lesions of epidemic encephalitis. *Archives of Neurology and Psychiatry*, 31, 685.
- 245) Freeman, J.M. (1969). The clinical spectrum and early diagnosis of Dawson's encephalitis. *Journal of Pediatrics*, 75, 590.
- 246) Agnarsdottir, G. (1977). Subacute sclerosing panencephalitis. In *Recent Advances in Clinical Virology*, ed., Waterson, A.P. Edinburgh: Churchill Livingstone, p. 21.
- 247) Jabbour, J.T., Duenas, D.A., Sever, J.L., Krebs, H.M.,

- and Horta-Barbosa, L. (1972). Epidemiology of subacute sclerosing panencephalitis (SSPE). *Journal of the American Medical Association*, 220, 959.
- 248) Canal, N., and Torck, P. (1964). An epidemiological study of sub-acute sclerosing leucoencephalitis in Belgium. *Journal of the Neurological Sciences*, 1, 380.
- 249) Cape, C.A., Martinez, J., Robertson, J.M., Hamilton, R., and Jabbour, J.T. (1973). Adult onset of subacute sclerosing panencephalitis. *Archives of Neurology*, 28, 124.
- 250) Gilden, D.H., Rorke, L.B., and Tanaka, R. (1975). Acute SSPE. *Archives of Neurology*, 32, 644.
- 251) Donner, M., Waltimo, O., Porras, J., Forsius, H., and Saukkonen, A.L. (1972). Subacute sclerosing panencephalitis as a cause of chronic dementia and relapsing brain disorder. *Journal of Neurology, Neurosurgery, and Psychiatry*, 35, 180.
- 252) Lowenthal, A., Moya, G., Poire, R., Macken, J., and De Smedt, R. (1972). Subacute sclerosing panencephalitis. A clinical and biological reappraisal. *Journal of the Neurological Sciences*, 15, 267.
- 253) Dubois-Dalcq, M., Coblenz, J.M., and Pleet, A.B.

(1974). Subacute sclerosing panencephalitis. Unusual nuclear inclusions and lengthy clinical course. Archives of Neurology, 31, 355.

254) Lombroso, C.T. (1968). Remarks on the EEG and movement disorder in SSPE. Neurology, 18, Part 2, 69.

255) Sourander, P., and Haltia, M. (1973). Some aspects of the pathology of subacute sclerosing panencephalitis (SSPE). Annals of Clinical Research, 5, 298.

256) Brody, J.A., and Detels, R. (1970). Subacute sclerosing panencephalitis: A zoonosis following aberrant measles. Lancet, ii, 500.

257) Kurent, J.E., Terasaki, P.I., and Sever, J.L. (1975). Association of HL-A histocompatibility antigen W-29 with SSPE. Archives of Neurology, 32, 494.

258) Whitaker, J.N., Sever, J.L., and King Engel, W. (1972). Subacute sclerosing panencephalitis in only one of identical twins. New England Journal of Medicine, 287, 864.

259) Bouteille, M., Fontaine, C., Verdrenne, Cl., et Delarue, J. (1965). Sur un cas d'encéphalite subaigue à inclusions. Etude anatomo- clinique et ultrastructurale. Revue Neurologique, 113, 454.

- 260) Tellez-Nagel, I., and Harter, D.H. (1966). Subacute sclerosing leukoencephalitis I. Clinico- pathological, electron microscopic and virological observations. *Journal of Neuropathology and Experimental Neurology*, 25, 560.
- 261) Shaw, C., Buchan, G., and Carlson, C.B. (1967). Myxovirus as a possible etiologic agent in subacute inclusion-body encephalitis. *New England Journal of Medicine*, 277, 511.
- 262) Herndon, R.M., and Rubinstein, L.J. (1968). Light and electron microscopy observations on the development of viral particles in the inclusions of Dawson's encephalitis (subacute sclerosing panencephalitis). *Neurology*, 18, Part 2, 8.
- 263) Shaw, C. (1968). Electron microscopic observations in subacute sclerosing panencephalitis. A supplementary report. *Neurology*, 18, Part 2, 144.
- 264) Connolly, J.H., Allen, I.V., Hurwitz, L.J., and Millar, J.H.D. (1967). Measles-virus antibody and antigen in subacute sclerosing panencephalitis. *Lancet*, i, 542.
- 265) Connolly, J.H. (1968). Additional data on measles virus antibody and antigen in subacute sclerosing panencephalitis. *Neurology*, 18, Part 2, 87.

- 266) Berman, P.H., Giles, J.P., and Krugman, S. (1968). Correlation of measles and subacute sclerosing panencephalitis. *Neurology*, 18, Part 2, 91.
- 267) Sever, J.T., and Zeman, W. (1968). Serological studies of measles and subacute sclerosing panencephalitis. *Neurology*, 18, Part 2, 95.
- 268) Jabbour, J.T., and Sever, J.L. (1968). Serum measles antibody titers in patients with subacute sclerosing panencephalitis, compared with parents and siblings. *Journal of Pediatrics*, 73, 905.
- 269) Brody, J.A., Detels, R., and Sever, J.L. (1972). Measles-antibody titres in sibships of patients with subacute sclerosing panencephalitis and controls. *Lancet*, i, 177.
- 270) Payne, F.E., Baublis, J.V., and Itabashi, H.H. (1969). Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. *New England Journal of Medicine*, 281, 585.
- 271) Horta-Barbosa, L., Fuccillo, D.A., London, W.T., Jabbour, J.T., Zeman, W., and Sever, J.L. (1969). Isolation of measles virus from brain cell cultures of two patients with subacute sclerosing panencephalitis. *Proceedings of the Society for Experimental Biology and*

Medicine, 132, 272.

272) ter Meulen, V., Katz, M., and Kackell, Y.M. (1973). Properties of SSPE virus; tissue culture and animal studies. *Annals of Clinical Research*, 5, 293.

273) Jabbour, J.T., Roane, J.A., and Sever, J.L. (1969). Studies of delayed dermal hypersensitivity in patients with subacute sclerosing panencephalitis. *Neurology*, 19, 929.

274) Ahmed, A., Strong, D.M., Sell, K.W., Thurman, G.B., Knudsen, R.C., Wistar, Jr., R., and Grace, W.R. (1974). Demonstration of a blocking factor in the plasma and spinal fluid of patients with subacute sclerosing panencephalitis. I. Partial characterization. *Journal of Experimental Medicine*, 139, 902.

275) Steele, R.W., Fuccillo, D.A., Hensen, S.A., Vincent, M.M., and Bellanti, J.A. (1976). Specific inhibitory factors of cellular immunity in children with subacute sclerosing panencephalitis. *Journal of Pediatrics*, 88, 56.

276) Dayan, A.D., and Stokes, M.I. (1972). Immune complexes and visceral deposits of measles antigens in subacute sclerosing panencephalitis. *British Medical Journal*, 2, 374.

277) Horta-Barbosa, L., Hamilton, R., Wittig, B., Fuccillo, D.A., Sever, J.L., and Vernon, M.L. (1971). Subacute

sclerosing panencephalitis: Isolation of suppressed measles virus from lymph node biopsies. *Science*, 173, 840.

278) Connolly, J.H., Haire, M., and Hadden, D.S.M. (1971). Measles immunoglobulins in subacute sclerosing panencephalitis. *British Medical Journal*, 1, 23.

279) Thomson, D., Connolly, J.H., Underwood, B.O., and Brown, F. (1975). A study of immunoglobulin M antibody to measles, canine distemper, and rinderpest viruses in sera of patients with subacute sclerosing panencephalitis. *Journal of Clinical Pathology*, 28, 543.

280) Vandvik, B., and Norrby, E. (1973). Oligoclonal Ig G antibody response in the central nervous system to different measles virus antigens in subacute sclerosing panencephalitis. *Proceedings of the National Academy of Sciences*, 70, 1060.

281) Vandvik, B. (1973). Immunopathological aspects in the pathogenesis of subacute sclerosing panencephalitis with special reference to the significance of the immune response in the central nervous system. *Annals of Clinical Research*, 5, 308.

282) Gardner, S.D. (1977). The new human papovaviruses: Their nature and significance. In *Recent Advances in Clinical Virology*, ed., Waterson, A.P. Edinburgh: Churchill Livingstone, p. 93.

- 283) Zu Rhein, G. (1969). Association of papova-virions with a human demyelinating disease (progressive multifocal leukoencephalopathy). *Progress in Medical Virology*, 11, 185.
- 284) Richardson, Jr., E.P. (1974). Our evolving understanding of progressive multifocal leukoencephalopathy. *Annals of the New York Academy of Sciences*, 230, 358.
- 285) Astrom, K.E., Mancall, E.L., and Richardson, Jr., E.P. (1958). Progressive multifocal leuko-encephalopathy. A hitherto unrecognized complication of chronic lymphatic leukaemia and Hodgkin's disease. *Brain*, 81, 93.
- 286) Zu Rhein, G., and Varakis, J. (1974). Progressive multifocal leukoencephalopathy in a renal-allograft recipient. *New England Journal of Medicine*, 291, 798.
- 287) Manz, H.J., Dinsdale, H.B., and Morrin, P.A.F. (1971). Progressive multifocal leukoencephalopathy after renal transplantation. Demonstration of papova-like virions. *Annals of Internal Medicine*, 75, 77.
- 288) Fermaglich, J., Hardman, J.M., and Earle, K.M. (1970). Spontaneous progressive multifocal leukoencephalopathy. *Neurology*, 20, 479.
- 289) Davies, J.A., Hughes, J.T., and Oppenheimer, D.R.

(1973). Richardson's disease (progressive multifocal leukoencephalopathy). *Quarterly Journal of Medicine*, 42, 481.

290) Brun, A., Nordenfeldt, E., and Kjellen, L. (1973). Aspects on the variability of progressive multifocal leukoencephalopathy. *Acta Neuropathologica*, 24, 232.

291) Hedley-Whyte, E.T., Smith, B.P., Tyler, H.R., Peterson, W.P. (1966). Multifocal leukoencephalopathy with remission and five year survival. *Journal of Neuropathology and Experimental Neurology*, 25, 107.

292) Kepes, J.J., Chou, S., and Price, Jr., L.W. (1975). Progressive multifocal leukoencephalopathy with 10 - year survival in a patient with nontropical sprue. *Neurology*, 25, 1006.

293) Zu Rhein, G.M., and Chou, S. (1965). Particles resembling papova viruses in human cerebral demyelinating disease. *Science*, 148, 1477.

294) Silverman, L., and Rubinstein, L.J. (1965). Electron microscopic observations on a case of progressive multifocal leukoencephalopathy. *Acta Neuropathologica*, 5, 215.

295) Padgett, B.L., Walker, D.L., Zu Rhein, G.M.,

Eckroade, R.J., and Dessel, B.H. (1971). Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet*, i, 1257.

296) Weiner, L.P., Narayan, O., Penney, J.B., Herndon, R.B., Feringa, E.R., Tourtellotte, W.W., and Johnson, R.T. (1973). Papovavirus of JC type in progressive multifocal leukoencephalopathy. *Archives of Neurology*, 29, 1.

297) Narayan, O., Penney, J.B., Johnson, R.T., Herndon, R.M., and Weiner, L.P. (1973). Etiology of progressive multifocal leukoencephalopathy. Identification of papovavirus. *New England Journal of Medicine*, 289, 1278.

298) Weiner, L.P., Herndon, R.M., Narayan, O., Johnson, R.T., Shah, K., Rubinstein, L.J., Preziosi, T.J., and Conley, F.K. (1972). Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy. *New England Journal of Medicine*, 286, 385.

299) Weiner, L.P., Herndon, R.M., Narayan, O., and Johnson, R.T. (1972). Further studies of a simian virus 40 - like virus isolated from human brain. *Journal of Virology*, 10, 147.

300) Gardner, S.D., Field, A.M., Coleman, D.V., and Hulme, B. (1971). New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet*, i, 1253.

- 301) Weiner, L.P., and Narayan, O. (1974). Virologic studies of progressive multifocal leukoencephalopathy. *Progress in Medical Virology*, 18, 229.
- 302) Walker, D.L., Padgett, B.L., Zu Rhein, G.M., Albert, A.E., and Marsh, R.F. (1973). Human papovavirus (J C) : Induction of brain tumors in hamsters. *Science*, 181, 674.
- 303) Meinke, W., Goldstein, D.A., and Smith, R.A. (1979). Simian virus 40 - related DNA sequences in a human brain tumor. *Neurology*, 29, 1590.
- 304) Padgett, B.L., and Walker, D.L. (1973). Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. *Journal of Infectious Diseases*, 127, 467.
- 305) Gardner, S.D. (1973). Prevalence in England of antibody to human polyomavirus (B.K.). *British Medical Journal*, 1, 77.
- 306) Shah, K.V., Daniel, R.W., and Warszawski, R.M. (1973). High prevalence of antibodies to BK Virus, an SV40-related papovavirus, in residents of Maryland. *Journal of Infectious Diseases*, 128, 784.
- 307) Shah, K.V. (1972). Evidence for an SV40-related papovavirus infection of man. *American Journal of*

Epidemiology, 95, 199.

308) Willoughby, E., Price, R.W., Padgett, B.L., Walker, D.L., and Dupont, B. (1980). Progressive multifocal leukoencephalopathy (PML): In vitro cell-mediated immune responses to mitogens and JC virus. *Neurology*, 30, 256.

309) Gibbs, Jr., C.J., and Gajdusek, D.C. (1972). Neurologic diseases of man with slow virus etiology. In *Membranes and Viruses in Immunopathology*, ed., Day, S.B., and Good, R.A. New York: Academic Press, p. 397.

310) Gajdusek, D.C., and Zigas, V. (1957). Degenerative disease of the central nervous system in New Guinea. The endemic occurrence of "kuru" in the native population. *New England Journal of Medicine*, 257, 974.

311) Klatzo, I., Gajdusek, D.C., and Zigas, V. (1959). Pathology of kuru. *Laboratory Investigation*, 8, 799.

312) Traub, R., Gajdusek, D.C., and Gibbs, Jr., C.J. (1977). Transmissible virus dementia: The relation of transmissible spongiform encephalopathy to Creutzfeldt-Jakob disease. In *Aging and Dementia*, ed., Smith., W.L., and Kinsbourne, M. New York: Spectrum Publications Inc., p. 91.

313) Hadlow, W.J. (1959). Scrapie and kuru. *Lancet*, ii, 289.

- 314) Gajdusek, D.C., Gibbs, Jr., C.J., and Alpers, M. (1966). Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature*, 209, 794.
- 315) Beck, E., Daniel, P.M., Alpers, M., Gajdusek, D.C., and Gibbs, Jr., C.J. (1966). Experimental "kuru" in chimpanzees. A pathological report. *Lancet*, ii, 1056.
- 316) Gibbs, Jr., C.J., and Gajdusek, D.C. (1972). Isolation and characterization of the subacute spongiform virus encephalopathies of man: Kuru and Creutzfeldt-Jakob disease. *Journal of Clinical Pathology*, 25, Suppl. (Roy. Coll. Path.), 6, 84.
- 317) Gajdusek, D.C., Rogers, N.G., Basnight, M., Gibbs, Jr., C.J., and Alpers, M. (1969). Transmission experiments with kuru in chimpanzees and the isolation of latent viruses from the explanted tissues of affected animals. *Annals of the New York Academy of Sciences*, 162, 529.
- 318) Gajdusek, D.C., Gibbs, Jr., C.J., Asher, D.M., Brown, P., Diwan, A., Hoffman, P., Nemo, G., Rohwer, R., and White, L. (1977). Precautions in medical care of, and in handling materials from, patients with transmissible virus dementia (Creutzfeldt-Jakob disease). *New England Journal of Medicine*, 297, 1253.
- 319) Grabow, J.D., Campbell, R.J., Okazaki, H., Schut, L.,

Zollman, P.E., and Kurland, L.T. (1976). A transmissible subacute spongiform encephalopathy in a visitor to the Eastern Highlands of New Guinea. *Brain*, 99, 637.

320) Benfante, R.J., Traub, R.D., Lim, K.A., Hooks, J., Gibbs, Jr., C.J., and Gajdusek, D.C. (1974). Immunological reactions in kuru. Attempts to demonstrate serological relationships between kuru and other known infectious agents. *American Journal of Tropical Medicine and Hygiene*, 23, 476.

321) Gajdusek, D.C., and Alpers, M. (1972). Genetic studies in relation to kuru. I. Cultural, historical, and demographic background. *American Journal of Human Genetics*, 24, suppl.: S1-S38.

322) Kitchin, F.D., Bearn, A.G., Alpers, M., and Gajdusek, D.C. (1972). Genetic studies in relation to kuru. III. Distribution of the inherited serum group-specific protein (Gc) phenotypes in New Guineans : An association of kuru and the Gc Ab Phenotype. *American Journal of Human Genetics*, 24, suppl.: S72-S85.

323) Simmons, R.T., Graydon, J.J., Gajdusek, D.C., Alpers M.P., and Hornabrook, R.W. (1972). Genetic studies in relation to kuru. II. Blood-group genetic patterns in kuru patients and populations of the Eastern Highlands of New Guinea. *American Journal of Human Genetics*, 24, suppl.:

S39-S71.

324) Kirschbaum, W.R. (1968). Jakob-Creutzfeldt Disease. New York: American Elsevier Publishing Company, Inc.

325) May, W.W. (1968). Creutzfeldt-Jakob disease. I. Survey of the literature and clinical diagnosis. *Acta Neurologica Scandinavica*, 44, 1.

326) Brown, P., Cathala, F., Sadowsky, D., and Gajdusek, D.C. (1979). Creutzfeldt-Jakob disease in France: II. Clinical characteristics of 124 consecutive verified cases during the decade 1968-1977. *Annals of Neurology*, 6, 430.

327) Roos, R., Gajdusek, D.C., Gibbs, Jr., C.J., (1973). The clinical characteristics of transmissible Creutzfeldt-Jakob disease. *Brain*, 96, 1.

328) Chou, S.M., and Martin, J.D. (1971). Kuru-plaques in a case of Creutzfeldt-Jakob disease. *Acta Neuropathologica*, 17, 150.

329) Hirano, A., Ghatak, N.R., Johnson, A.B., Partnow, M.J., and Gomori, A.J. (1972). Argentophilic plaques in Creutzfeldt-Jakob disease. *Archives of Neurology*, 26, 530.

330) Daniel, P.M. (1972). Creutzfeldt-Jakob disease. *Journal of Clinical Pathology*, 25, Suppl. (Roy. Coll. Path.),

6, 97.

331) Gibbs, Jr., C.J., Gajdusek, D.C., Asher, D.M., Alpers, M.P., Beck, E., Daniel, P.M., and Matthews, W.B. (1968).

Creutzfeldt-Jakob disease (spongiform encephalopathy):
Transmission to the chimpanzee. *Science*, 161, 388.

332) Gibbs, Jr., C.J., and Gajdusek, D.C. (1969).

Infection as the etiology of spongiform encephalopathy
(Creutzfeldt-Jakob disease). *Science*, 165, 1023.

333) Brown, P., and Cathala, F. (1979). Creutzfeldt-

Jakob disease in France: I. Retrospective study of the
Paris area during the ten-year period 1968-1977. *Annals of
Neurology*, 5, 189.

334) Brown, P., Cathala, F., and Gajdusek, D.C. (1979).

Creutzfeldt-Jakob disease in France: III. Epidemiological
study of 170 patients dying during the decade 1968-1977.
Annals of Neurology, 6, 438.

335) Masters, C.L., Harris, J.O., Gajdusek, D.C., Gibbs,
Jr., C.J., Bernoulli, C., and Asher, D.M. (1979).

Creutzfeldt-Jakob disease: Patterns of worldwide
occurrence and the significance of familial and sporadic
clustering. *Annals of Neurology*, 5, 177.

336) Neugut, R.H., Neugut, A.I., Kahana, E., Stein, Z.,
and Alter, M. (1979). Creutzfeldt-Jakob disease:

Familial clustering among Libyan-born Israelis. *Neurology*, 29, 225.

337) Kovanen, J., Tiilikainen, A., and Haltia, M. (1980). Histocompatibility antigens in familial Creutzfeldt-Jakob disease. *Journal of the Neurological Sciences*, 45, 317.

338) Cathala, F., Chatelain, J., Brown, P., Dumas, M., and Gajdusek, D.C. (1980). Familial Creutzfeldt-Jakob disease. Autosomal dominance in 14 members over 3 generations. *Journal of the Neurological Sciences*, 47, 343.

339) Galvez, S., Masters, C., and Gajdusek, D.C. (1980). Descriptive epidemiology of Creutzfeldt-Jakob disease in Chile. *Archives of Neurology*, 37, 11.

340) Kahana, E., Alter, M., Braham, J., and Sofer, D. (1974). Creutzfeldt-Jakob disease: Focus among Libyan Jews in Israel. *Science*, 183, 90.

341) Brown, P., Hooks, J., Roos, R., Gajdusek, D.C., and Gibbs, Jr., C.J. (1972). Attempt to identify the agent for Creutzfeldt-Jakob disease by CF antibody relationship to known viruses. *Nature New Biology*, 235, 149.

342) Matthews, W.B. (1975). Epidemiology of Creutzfeldt-Jakob disease in England and Wales. *Journal of Neurology, Neurosurgery, and Psychiatry*, 38, 210.

343) Mayer, V., Orolin, D., Mitrova, E., and Lehotsky, T. (1978). Transmissible virus dementia. I. An unusual space and time clustering of Creutzfeldt-Jakob disease and of other organic presenile dementia cases. *Acta Virologica*, 22, 146.

344) Corsellis, J.A.N. (1979). On the transmission of dementia. A personal view of the slow virus problem. *British Journal of Psychiatry*, 134, 553.

345) Duffy, P., Wolf, J., Collins, G., DeVoe, A.G., Streeten, B., and Cowen, D. (1974). Possible person-to-person transmission of Creutzfeldt-Jakob disease. *New England Journal of Medicine*, 299, 692.

346) Bernoulli, C., Siegfried, J., Baumgartner, G., Regli, F., Rabinowicz, Gajdusek, D.C., and Gibbs, Jr., C.J. (1977). Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet*, i, 478.

347) Bobowick, A.R., Brody, J.A., Matthews, M.R., Roos, R., and Gajdusek, D.C. (1973). Creutzfeldt-Jakob disease: A case control study. *American Journal of Epidemiology*, 98, 381.

348) Herzberg, L., Herzberg, B.N., Gibbs, Jr., C.J., Sullivan, W., Amyx, H., and Gajdusek, D.C., (1974). Creutzfeldt-Jakob disease: Hypothesis for high incidence

in Libyan Jews in Israel. *Science*, 186, 848.

349) Matthews, W.B., Campbell, M., Hughes, J.T., and Tomlinson, A.H. (1979). Creutzfeldt-Jakob disease and ferrets. *Lancet*, i, 828.

350) Wisniewski, K., Jervis, G.A., Moretz, R.C., and Wisniewski, H.M. (1979). Alzheimer neurofibrillary tangles in diseases other than senile and presenile dementia. *Annals of Neurology*, 5, 288.

351) De Boni, U., and Crapper, D.R. (1978). Paired helical filaments of the Alzheimer type in cultured neurones. *Nature*, 271, 566.

352) Crapper, D.R., and De Boni, U. (1978). Brain aging and Alzheimer's disease. *Canadian Psychiatric Association Journal*, 23, 229.

353) Fraser, H., and Bruce, M. (1973). Argrophilic plaques in mice inoculated with scrapie from particular sources. *Lancet*, i, 617.

354) Wisniewski, H.M., Bruce, M.E., and Fraser, H. (1975). Infectious etiology of neuritic (senile) plaques in mice. *Science*, 190, 1108.

355) McDermott, J.R., Fraser, H., and Dickinson, A.G. (1978).

Reduced choline-acetyltransferase activity in scrapie mouse brain. *Lancet*, ii, 318.

356) Grunnet, M.L. (1975). Nuclear bodies in Creutzfeldt-Jakob and Alzheimer's diseases. *Neurology*, 25, 1091.

357) Goudsmit, J., Morrow, C.H., Asher, D.M., Yanagihara, R.T., Masters, C.L., Gibbs, Jr., C.J., and Gajdusek, D.C. (1980). Evidence for and against the transmissibility of Alzheimer disease. *Neurology*, 30, 945.

358) Johnson, R.T., Narayan, O., and Clements, J. (1978). Varied role of viruses in chronic neurologic diseases. In *Persistent Viruses*, ed., Stevens, J.G., Todaro, G.J., and Fox, C.F. London: Academic Press, p. 551.

359) Jack, I. (1974). Persistent infections with herpesviruses. In *Progress in Medical Virology*, 18, 160.

360) Smith, J.W. (1978). Herpesviruses. In *Human Diseases caused by Viruses*, ed., Rothschild, H., Allison, Jr., F., and Howe, C. New York: Oxford University Press, p. 149.

361) Koprowski, H. (1978). Possible role of herpes virus in the chronic CNS diseases. In *Persistent Viruses*, ed., Stevens, J.G., Todaro, G.J., and Fox, C.F. London: Academic Press, p. 691.

- 362) Rose, F.C., and Symonds, C.P. (1960). Persistent memory defect following encephalitis. *Brain*, 83, 195.
- 363) Drachman, D.A., and Adams, R.D. (1962). Herpes simplex and acute inclusion-body encephalitis. *Archives of Neurology*, 7, 61.
- 364) Libikova, H., Pogady, J., Wiedermann, V., and Breier, S. (1975). Search for herpetic antibodies in the cerebrospinal fluid in senile dementia and mental retardation. *Acta Virologica*, 19, 493.
- 365) Libikova, H., Pogady, J., Rajcani, J., Skodacek, I., Ciampor, F., and Kocisova, M. (1979). Latent herpesvirus hominis I in the central nervous system of psychotic patients. *Acta Virologica*, 23, 231.
- 366) Lycke, E., Norrby, R., and Roos, B. (1974). A serological study on mentally ill patients. With particular reference to the prevalence of herpes virus infections. *British Journal of Psychiatry*, 124, 273.
- 367) Lord, A., Sutton, R.N.P., Baker, A.A., and Hussein, S.A.M. (1978). Serological studies in the elderly. *Age and Ageing*, 7, 116.
- 368) Whalley, L.J., Urbaniak, S.J., Darg, C., Peutherer, J. F., and Christie, J.E. (1980). Histocompatibility antigens and antibodies to viral and other antigens in Alzheimer

pre-senile dementia. *Acta Psychiatrica Scandinavica*, 61, 1.

369) Sequiera, L.W., Jennings, L.C., Carrasco, L.H., Lord, M.A., Curry, A., and Sutton, R.N.P. (1979). Detection of herpes-simplex viral genome in brain tissue. *Lancet*, ii, 609.

370) Weller, T.H. (1970). Cytomegaloviruses: The difficult years. *The Journal of Infectious Diseases*, 122, 532.

371) Weller, T.H. (1971). The cytomegaloviruses: Ubiquitous agents with protean clinical manifestations. *New England Journal of Medicine*, 285, 203.

372) Dudgeon, J.A. (1971). Cytomegalovirus infection. *Archives of Disease in Childhood*, 46, 581.

373) Stern, H. (1972). Cytomegalovirus: A cause of persistent latent infection. *Journal of Clinical Pathology*, 25, Suppl. (Roy. Coll. Path.), 6, 34.

374) Stern, H. (1972). Cytomegalovirus and EB virus infections of the liver. *British Medical Bulletin*, 28, 180.

375) Stern, H. (1968). Isolation of cytomegalovirus and clinical manifestations of infection at different ages. *British Medical Journal*, 1, 665.

- 376) Plummer, G. (1973). Cytomegaloviruses of man and animals. In *Progress in Medical Virology*, Vol. 15, ed. Melnick, J.L. Basel: Karger, p. 92.
- 377) Anon. (1974). Cytomegalovirus again. *British Medical Journal*, 2, 593.
- 378) Gold, E., and Nankervis, G.A. (1976). Cytomegalovirus. In *Viral Infections of Humans*, ed., Evans, A.S. New York: John Wiley and Sons, p. 143.
- 379) Anon. (1977). Cytomegalovirus in adults. *Lancet*, 2, 541.
- 380) Huang, E., and Pagano, J.S. (1977). Comparative diagnosis of cytomegaloviruses: New approach. In *Comparative Diagnosis of Viral Diseases*. Vol. 1, ed. Kurstak, E., and Kurstak, C. New York: Academic Press, p. 241.
- 381) Stern, H., and Elek, S.D. (1965). The incidence of infection with cytomegalovirus in a normal population. A serological study in Greater London. *J. Hyg., Camb.*, 63, 79.
- 382) Jack, I, and McAuliffe, K.C. (1968). Sero-epidemiological study of cytomegalovirus infections in Melbourne children and some adults. *Medical Journal of Australia*, 1, 206.

- 383) Craighead, J.E. (1969). Immunologic response to cytomegalovirus infection in renal allograft recipients. *American Journal of Epidemiology*, 90, 506.
- 384) Schauf, V., Strelkauskas, A.J., and Deveikis, A. (1976). Alteration of lymphocyte subpopulations with cytomegalovirus infection in infancy. *Clin. Exp. Immunol.*, 26, 478.
- 385) Rytel, M.W., and Balay, J. (1976). Cytomegalovirus infection and immunity in renal allograft recipients: Assessment of the competence of humoral immunity. *Infection and Immunity*, 13, 1633.
- 386) Oill, P.A., Fiala, M., Schofferman, J., Byfield, P.E., and Guze, L.B. (1977). Cytomegalovirus mononucleosis in a healthy adult. Association with hepatitis, secondary Epstein-Barr virus antibody response and immunosuppression. *The American Journal of Medicine*, 62, 413.
- 387) Rytel, M.W., Aguilar-Torres, F.G., Balay, J., and Heim, L.R. (1978). Assessment of the status of cell-mediated immunity in cytomegalovirus-infected renal allograft recipients. *Cellular Immunology*, 37, 31.
- 388) Gehrz, R.C., Marker, S.C., and Balfour, H.H. (1977). Specific cell-mediated immune defect in congenital cytomegalovirus infection. *Lancet*, i, 811.

- 389) Gehrz, R.C., Marker, S.C., Knorr, S.O., Kalis, J.M., and Balfour, Jr., H.H. (1977). Specific cell-mediated immune defect in active cytomegalovirus infection of young children and their mothers. *Lancet*, ii, 844.
- 390) Starr, S.E., Tolpin, M.D., Friedman, H.M., Plotkin, S.A., and Paucker, K. (1977). Immune responses in children with congenital cytomegalovirus and their mothers. *Lancet*, ii, 1357.
- 391) Rola-Pleszczynski, M., Frenkel, L.D., Fuccillo, D.A., Hensen, S.A., Vincent, M.M., Reynolds, D.W., Stagno, S., and Bellanti, J.A. (1977). Specific impairment of cell-mediated immunity in mothers of infants with congenital infection due to cytomegalovirus. *The Journal of Infectious Diseases*, 135, 386.
- 392) Fiorilli, M., Sirianni, M.C., Spaziani, A., Aiuti, F., and Pana, A. (1978). Immune responses in cytomegalovirus infection. *Lancet*, i, 780.
- 393) Pollard, R.B., Rand, K.H., Arvin, A.M., and Merigan, T.C. (1978). Cell-mediated immunity to cytomegalovirus infection in normal subjects and cardiac transplant patients. *Journal of Infectious Diseases*, 137, 541.
- 394) Agatsuma, Y., Fitzpatrick, P., Kaul, A., Kaul, R., Kohli, R., Paves, A.V., and Ogra, P.L. (1979). Cell-mediated

immunity to cytomegalovirus in immunocompromised patients: Effect of radiation or chemotherapy. *Infection and Immunity*, 26, 217.

395) Stern, H., and Tucker, S.M. (1973). Prospective study of cytomegalovirus infection in pregnancy. *British Medical Journal*, 1, 268.

396) Weller, T.H., and Hanshaw, J.B. (1962). Virologic and clinical observations on cytomegalic inclusion disease. *New England Journal of Medicine*, 266, 1233.

397) Medearis, Jr., D.N. (1964). Observations concerning human cytomegalovirus infection and disease. *Bulletin of the Johns Hopkins Hospital*, 114, 181.

398) Stern, H., Elek, S.D., Booth, J.C., and Fleck, D.G. (1969). Microbial causes of mental retardation. The role of prenatal infections with cytomegalovirus, rubella virus and toxoplasma. *Lancet*, ii, 443.

399) Hanshaw, J.B. (1971). Congenital cytomegalovirus infection: A fifteen year perspective. *Journal of Infectious Diseases*, 123, 555.

400) Reynolds, D.W., Stagno, S., Stubbs, K.G., Dahle, A.J., Livingston, M.M., Samuel, M.A., Saxon, S.S., and Alford, C.A. (1974). Inapparent congenital cytomegalovirus

infection with elevated cord Ig M levels. Causal relation with auditory and mental deficiency. *New England Journal of Medicine*, 290, 291.

401) Whitley, R.J., and Alford, Jr., C.A. (1979). Chronic intrauterine and perinatal infections. In *Antiviral Agents and Viral Diseases of Man*, ed. Galasso, G.J., Merigan, T.C., and Buchanan, R.A. New York: Raven Press, p. 558.

402) Klemola, E., and Kaariainen, L. (1965). Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. *British Medical Journal*, 2, 1099.

403) Anderson, J.P., and Stern, H. (1966). Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. *British Medical Journal*, 1, 672.

404) Lamb, S.G., and Stern, H. (1966). Cytomegalovirus mononucleosis with jaundice as presenting sign. *Lancet*, ii, 1003.

405) Klemola, E., von Essen, R., Wager, O., Haltia, K., Koivuniemi, A., and Salmi, I. (1969). Cytomegalovirus mononucleosis in previously healthy individuals. Five new cases and follow-up of 13 previously published cases. *Annals of Internal Medicine*, 71, 11.

406) Hanshaw, J.B., Betts, R.F., Simon, G., and Boynton,

R.C. (1965). Acquired cytomegalovirus infection.

Association with hepatomegaly and abnormal liver-function tests. *New England Journal of Medicine*, 272, 602.

407) Rasanen, V., and Saikku, P. (1967). Cytomegalovirus hepatitis. *Lancet*, ii, 772.

408) Evans, D.B., Millard, P.R., and Herbertson, B.M. (1968). Hepatic dysfunction associated with renal transplantation. *Lancet*, ii, 929.

409) Carter, A.R. (1968). Cytomegalovirus disease presenting as hepatitis. *British Medical Journal*, 2, 786.

410) Hill, Jr., R.B., Rowlands, Jr., D.T., and Rifkind, D. (1964). Infectious pulmonary disease in patients receiving immunosuppressive therapy for organ transplantation. *New England Journal of Medicine*, 271, 1021.

411) Coombs, R.R.H. (1968). Cytomegalic inclusion-body disease associated with acquired autoimmune haemolytic anaemia. *British Medical Journal*, 1, 743.

412) Zuelzer, W.W., Mastrangelo, R., Stulberg, C.S., Poulik, M.D., Page, R.H., and Thompson, R.I. (1970). Autoimmune haemolytic anaemia. Natural history and viral-immunologic interactions in childhood. *American Journal of Medicine*, 49, 80.

- 413) Paloheimo, J.A., von Essen, R., Klemola, E., Kaariainen, L., and Siltanen, P. (1968). Subclinical cytomegalovirus infections and cytomegalovirus mononucleosis after open heart surgery. *American Journal of Cardiology*, 22, 624.
- 414) Lang, D.J., Scolnick, E.M., and Willerson, J.T. (1968). Association of cytomegalovirus infection with the postperfusion syndrome. *New England Journal of Medicine*, 278, 1147.
- 415) Lang, D.J., and Hanshaw, J.B. (1969). Cytomegalovirus infection and the postperfusion syndrome. Recognition of primary infections in four patients. *New England Journal of Medicine*, 280, 1145.
- 416) Foster, K.M., and Jack, I. (1969). A prospective study of the role of cytomegalovirus in post-transfusion mononucleosis. *New England Journal of Medicine*, 280, 1311.
- 417) Diosi, P., Moldovan, E., and Tomescu, N. (1969). Latent cytomegalovirus infection in blood donors. *British Medical Journal*, 2, 660.
- 418) Hanshaw, J.B., and Weller, T.H. (1961). Urinary excretion of cytomegaloviruses by children with generalized neoplastic disease. *Journal of Pediatrics*, 58, 305.
- 419) Bodey, G.P., Wertlake, P.T., Douglas, G., and Levin,

R.H., (1965). Cytomegalic inclusion disease in patients with acute leukaemia. *Annals of Internal Medicine*, 62, 899.

420) Cangir, A., and Sullivan, M.P. (1966). The occurrence of cytomegalovirus infections in childhood leukemia. *Journal of the American Medical Association*, 195, 616.

421) Duvall, C.P., Casazza, A.R., Grimley, P.M., Carbone, P.P., and Rowe, W.P. (1966). Recovery of cytomegalovirus from adults with neoplastic disease. *Annals of Internal Medicine*, 64, 531.

422) Sullivan, M.P., Hanshaw, J.B., Cangir, A., and Butler, J.J. (1968). Cytomegalovirus complement-fixation antibody levels of leukemic children. *Journal of the American Medical Association*, 206, 569.

423) Rifkind, D. (1965). Cytomegalovirus infection after renal transplantation. *Archives of Internal Medicine*, 116, 554.

424) Kanich, R.E., and Craighead, J.E. (1966). Cytomegalovirus infection and cytomegalic inclusion disease in renal homotransplant recipients. *American Journal of Medicine*, 40, 874.

425) Craighead, J.E., Hanshaw, J.B., and Carpenter, C.B.

(1967). Cytomegalovirus infection after renal allotransplantation. *Journal of the American Medical Association*, 201, 725.

426) Rifkind, D., Goodman, N., and Hill, R.B. (1967). The clinical significance of cytomegalovirus infection in renal transplant recipients. *Annals of Internal Medicine*, 66, 1116.

427) Kerzel Andersen, H., and Spencer, E.S. (1969). Cytomegalovirus infection among renal allograft recipients. *Acta Medica Scandinavica*, 186, 7.

428) Suwansirikul, S., Rao, N., Dowling, J.N., and Ho, M. (1977). Primary and secondary cytomegalovirus infection. Clinical manifestations after renal transplantation. *Archives of Internal Medicine*, 137, 1026.

429) Lopez, C., Simmons, R.L., Mauer, S.M., Najarian, J.S., and Good, R.A. (1974). Association of renal allograft rejection with virus infections. *American Journal of Medicine*, 56, 280.

430) Anon. (1978). Should renal-transplant patients be screened for C.M.V.? *Lancet*, 2, 770.

431) Balfour, H.H. (1979). Cytomegalovirus. The troll of transplantation. *Archives of Internal Medicine*, 139, 279.

432) Ironside, A.G., and Tobin, J., O'H. (1967).

Cytomegalovirus infection in the adult. *Lancet*, ii, 615.

433) Klemola, E., Weckman, N., Haltia, K., and Kaariainen,

L. (1967). The Guillain-Barré syndrome associated with acquired cytomegalovirus infection. *Acta Medica Scandinavica*, 181, 603.

434) Leonard, J.C., and Tobin, J. O'H. (1971). Polyneuritis associated with cytomegalovirus infections. *Quarterly Journal of Medicine, New Series*, 159, 435.

435) Schmitz, H., and Enders, G. (1977). Cytomegalovirus as a frequent cause of Guillain-Barré syndrome. *Journal of Medical Virology*, 1, 21.

436) Dowling, P., Menonna, J., and Cook, S. (1977). Cytomegalovirus complement fixation antibody in Guillain-Barré syndrome. *Neurology*, 27, 1153.

437) Duchowny, M., Caplan, L., and Siber, G. (1979). Cytomegalovirus infection of the adult nervous system. *Annals of Neurology*, 5, 458.

438) Perham, T.G.M., Caul, E.O., Clarke, S.K.R., and Gibson, A.G.F. (1971). Cytomegalovirus meningo-encephalitis. *British Medical Journal*, 2, 50.

- 439) Chin, W., Magoffin, R., Frierson, J.G., and Lennette, E.H. (1973). Cytomegalovirus infection. A case with meningoencephalitis. *Journal of the American Medical Association*, 225, 740.
- 440) Dorfman, L.J. (1973). Cytomegalovirus encephalitis in adults. *Neurology*, 23, 136.
- 441) Phillips, C.A., Fanning, W.L., Gump, D.W., Phillips, C.F. (1977). Cytomegalovirus encephalitis in immunologically normal adults. Successful treatment with vidarabine. *Journal of the American Medical Association*, 238, 2299.
- 442) Yanagisawa, N., Toyokura, Y., and Shiraki, H. (1975). Double encephalitis with herpes simplex virus and cytomegalovirus in an adult. *Acta Neuropathologica*, 33, 153.
- 443) Schneck, S.A. (1965). Neuropathological features of human organ transplantation I. Probable cytomegalovirus infection. *J. Neuropathol. Exp. Neurol.*, 24, 415.
- 444) Lussier, G. (1975). Murine cytomegalovirus-induced cerebral calcification. *American Journal of Pathology*, 80, 555.
- 445) Wroblewska, Z., Gilden, D., Devlin, M., Huang, E., Rorke, L.B., Hamada, T., Furukawa, T., Cummins, L.,

Kalter, S., and Koprowski, H. (1979). Cytomegalovirus isolation from a chimpanzee with acute demyelinating disease after inoculation of multiple sclerosis brain cells. *Infection and Immunity*, 25, 1008.

446) Bodmer, W.F. (1972). Evolutionary significance of the HLA system. *Nature*, 237, 139.

447) McDevitt, H.O., and Bodmer, W.F. (1972). Histocompatibility antigens, immune responsiveness and susceptibility to disease. *The American Journal of Medicine*, 52, 1.

448) McDevitt, H.O., and Bodmer, W.F. (1974). HLA, immune response genes, and disease. *Lancet*, i, 1269.

449) Bodmer, W.F., and Bodmer, J.G. (1974). The HLA histocompatibility antigens and disease. In *Advanced Medicine Symposium*, 10. Royal College of Physicians of London. London: Pitman Medical, p. 157.

450) McDevitt, H.O. (1976). Immune response genes in relation to disease. In *The Role of Immunological Factors in Infectious, Allergic and Autoimmune Processes*, ed. Beers, Jr., R.F., and Basset, E. New York: Raven Press, p. 255.

451) McConnell, I., Munro, A., and Waldmann, H. (1981). Chapter 18. HLA and Disease. In *The Immune System* (2nd ed.). Oxford, London, Edinburgh: Blackwell Scientific

Publications, p. 283.

452) Dausset, J., and Svejgaard, A. (ed). (1977). HLA and Disease. Copenhagen: Munksgaard.

453) McMichael, A., and McDevitt, H. (1977). The association between the HLA system and disease. In Progress in Medical Genetics, 2, ed. Steinberg, A.G., Bearn, A.G., Motulsky, A.G., and Childs, B., p. 39.

454) Zinkernagel, R.M. (1981). Chapter 6. Cellular Immune Response to Intracellular Parasites and the Biological Role of Polymorphic Major Transplantation Antigens. In Immunological Recognition and Effector Mechanisms in Infectious Diseases, ed., Torrigiani, G., and Bell, R. Basel: Schwabe and Co., p. 69.

455) Festenstein, H., and Démant, P. (1978). The human major histocompatibility system. In HLA and H-2. London: Edward Arnold, p. 16.

456) McConnell, I., Munro, A., and Waldmann, H. (1981). Chapter 12. The Biology of the Major Histocompatibility System - M H S Immune Response Genes. In The Immune System, (2nd ed.). Oxford, London, Edinburgh: Blackwell Scientific Publications, p. 191.

457) Gill, T.J., Cramer, D.V., and Kunz, H.W. (1978). The

major histocompatibility complex - Comparison in the mouse, man, and the rat. American Journal of Pathology, 90, 737.

458) Parish, C.R., Sia, D.Y., and Rylatt, D.B. (1981).

Presence of receptors for self MHC antigens on non-T cells. Immunology Today, 2, 9, September, p. 172.

459) Festenstein, H., and Déchant, P. (1978). Table A1.5.

Complete listing of recognized HLA specificities. In HLA and H-2. London: Edward Arnold, p. 184.

460) Payne, R. (1977). The HLA complex: Genetics and

implications in the immune response. In HLA and Disease, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 20.

461) Barnstable, C.J., Jones, E.A., and Crumpton, M.J.

(1978). Isolation, structure and genetics of HLA-A, -B, -C and -DRw (Ia) antigens. British Medical Bulletin, Vol. 34, No. 3, p. 241.

462) Joysey, V.C., and Wolf, E. (1978). HLA-A, -B and -C

antigens, their serology and cross-reaction. British Medical Bulletin, Vol. 34, No. 3, p. 217.

463) Bodmer, W.F., and Bodmer, J.G. (1978). Evolution and

function of the HLA system. British Medical Bulletin, Vol. 34, No. 3. p. 309.

464) Bradley, B.A., and Festenstein, H. (1978). Cellular typing. British Medical Bulletin, Vol. 34, No. 3, p. 223.

465) Bodmer, J.G. (1978). Ia antigens. Definition of the HLA-DRw specificities. British Medical Bulletin, Vol. 34, No. 3, p. 233.

466) Shreffler, D.C. (1977). The H-2 model: Genetic control of immune functions. In HLA and Disease, ed. Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 32.

467) Munro, A., and Waldmann, H. (1978). The major histocompatibility system and the immune response. British Medical Bulletin, Vol. 34, No. 3, p. 253.

468) Lachmann, P.J., and Hobart, M.J. (1978). Complement genetics in relation to HLA. British Medical Bulletin, Vol. 34, No. 3, p. 247.

469) Morris, P.J., Batchelor, J.R., and Festenstein, H. (1978). Matching for HLA in transplantation. British Medical Bulletin, Vol. 34, No. 3, p. 259.

470) Smith, G.S., and Walford, R.L. (1977). Influence of the main histocompatibility complex on ageing in mice. Nature, 270, 727.

- 471) Dick, H.M. (1978). HLA and disease. Introductory review . British Medical Bulletin, Vol. 34, No. 3, p. 271.
- 472) Festenstein, H., and Démant, P. (1978). The MHS and disease. In HLA and H-2. London: Edward Arnold, p. 161.
- 473) Svejgaard, A., and Ryder, L.P. (1977). Associations between HLA and disease. In HLA and Disease, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 46.
- 474) Dausset, J. (1977). Clinical implications (nosology, diagnosis, prognosis and preventive therapy). In HLA and Disease, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 296.
- 475) Bodmer, W., and Thomson, G. (1977). Population genetics and evolution of the HLA system. In HLA and Disease, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 280.
- 476) Mourant, A.E., Kopeć, A.C., and Domaniewska - Sobczak, K. (1978). Blood Groups and Diseases. Oxford: Oxford University Press.
- 477) Mourant, A.E., Kopeć, A.C., and Domaniewska - Sobczak, K. (1976). The ABO blood groups. The Distribution of the Human Blood Groups and Other Polymorphisms. London:

Oxford University Press, p. 4.

478) Dodd, B.E., and Lincoln, P.J. (1975). Antigens and antibodies. In Blood Group Topics. London: Edward Arnold, p. 11.

479) McKusick, V.A., and Ruddle, F.H. (1977). The status of the gene map of the human chromosomes. Science, 196, 390.

480) Dodd, B.E., and Lincoln, P.J. (1975). Haemolytic disease of the newborn. In Blood Group Topics. London: Edward Arnold, p. 93.

481) Dodd, B.E., and Lincoln, P.J. (1975). Incompatibility. In Blood Group Topics. London: Edward Arnold, p. 85.

482) Clarke, C.A. (1961). Blood groups and disease. Progress in Medical Genetics, 1, 81.

483) Fraser Roberts, J.A. (1959). Some associations between blood groups and disease. British Medical Bulletin, 15, 129.

484) Mourant, A.E. (1973). Associations between hereditary blood factors and diseases. Bulletin of the World Health Organization, 49, 93.

- 485) Aird, I., Bentall, H.H., and Fraser Roberts, J.A. (1953). A relationship between cancer of stomach and the ABO blood groups. *British Medical Journal*, 1, 799.
- 486) Aird, I., Bentall, H.H., Mehigan, J.A., and Fraser Roberts, J.A. (1954). The blood groups in relation to peptic ulceration and carcinoma of colon, rectum, breast, and bronchus. An association between the ABO groups and peptic ulceration. *British Medical Journal*, 2, 315.
- 487) Clarke, C.A., Cowan, W.K., Wyn Edwards, J., Howel-Evans, A.W., McConnell, R.B., Woodrow, J.C., and Sheppard, P.M. (1955). The relationship of the ABO blood groups to duodenal and gastric ulceration. *British Medical Journal*, 2, 643.
- 488) Clarke, C.A., Wyn Edwards, J., Haddock, D.R.W., Howel-Evans, A.W., McConnell, R.B., and Sheppard, P.M. (1956). ABO blood groups and secretor character in duodenal ulcer. *British Medical Journal*, 2, 725.
- 489) Dodd, B.E., and Lincoln, P.J. (1975). The structure of various blood group systems. In *Blood Group Topics*. London: Edward Arnold, p. 60.
- 490) MacDonald, J.L., Roberts, D.F., Shaw, D.A., and Saunders, M. (1976). Blood groups and other polymorphisms in multiple sclerosis. *Journal of Medical Genetics*, 13, 30.

- 491) Dodd, B.E., and Lincoln, P.J. (1975). The general principles underlying blood grouping techniques. In Blood Group Topics. London: Edward Arnold, p. 40.
- 492) Bradstreet, C.M., Patricia, and Taylor, C.E.D. (1962). Technique of complement fixation test applicable to the diagnosis of virus diseases. Mon. Bull. Minist. Hlth., 21, 96.
- 493) Kopeć, A.C. (1970). ABO distributions in Great Britain. IX. Leeds. In The Distribution of the Blood Groups in the United Kingdom. London: Oxford University Press, p. 52. (area numbers 4-27, and 29-32).
- 494) Kopeć, A.C. (1970). The distribution of rhesus negatives in Great Britain. IX. Leeds. In The Distribution of the Blood Groups in the United Kingdom. London: Oxford University Press, p. 117. (area numbers 4-27, and 29-32).
- 495) Corsellis, J.A.N. (1962). Mental Illness and the Ageing Brain. London: Oxford University Press.
- 496) Marsden, C.D. (1978). The diagnosis of dementia. In Studies in Geriatric Psychiatry, ed., Isaacs, A.D., and Post, F. Chichester: John Wiley and Sons. p. 95.
- 497) Terry, R.D., (1978). Aging, senile dementia, and

Alzheimer's disease. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 11.

498) Hachinski, V.C., Lassen, N.A., and Marshall, J. (1974). Multi-infarct dementia. A cause of mental deterioration in the elderly. *Lancet*, ii, 207.

499) Rothschild, D. (1941). The clinical differentiation of senile and arteriosclerotic psychoses. *American Journal of Psychiatry*, 98, 324.

500) Birkett, D.P. (1972). The psychiatric differentiation of senility and arteriosclerosis. *British Journal of Psychiatry*, 120, 321.

501) Roth, M. (1978). Diagnosis of senile and related forms of dementia. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 71.

502) Hachinski, V.C., Iliff, L.D., Zilhka, E., Du Boulay, G.H., McAllister, V.L., Marshall, J., Russell, R.W.R., and Symon, L. (1975). Cerebral blood flow in dementia. *Archives of Neurology*, 32, 632.

503) Hachinski, V. (1978). Cerebral blood flow: Differentiation of Alzheimer's disease from multi-infarct

dementia. In *Alzheimer's Disease: Senile Dementia and Related Disorders*, ed., Katzman, R., Terry, R.D., and Bick, K.L. New York: Raven Press, p. 97.

504) Slater, E., and Roth, M. (1969). In *Clinical Psychiatry*, (3rd ed.). London: Bailliere, Tindall, and Cassell, p. 617.

505) Sim, M. (1979). Early diagnosis of Alzheimer's disease. In *Alzheimer's Disease. Early Recognition of Potentially Reversible Deficits*, ed., Glen, A.I.M., and Whalley, L.J. Edinburgh: Churchill Livingstone, p. 78.

506) Sim, M., and Sussman, I. (1962). Alzheimer's disease: Its natural history and differential diagnosis. *Journal of Nervous and Mental Diseases*, 135, 489.

507) Sim, M., Turner, E., and Smith, W.T. (1966). Cerebral biopsy in the investigation of presenile dementia I. Clinical aspects. *British Journal of Psychiatry*, 112, 119.

508) Todorov, A.B., Go, R.C.P., Constantinidis, J., and Elston, R.C. (1975). Specificity of the clinical diagnosis of dementia. *Journal of the Neurological Sciences*, 26, 81.

509) Vogel, F. (1970). ABO blood groups and disease. *The American Journal of Human Genetics*, 22, 464.

510) Buckwalter, J.A., and Knowler, L.A. (1958). Blood donor controls for blood group disease researches. The American Journal of Human Genetics, 10, 164.

511) Yarnell, J.W.G., St. Leger, A.S., Balfour, I.C., and Russell, R.B. (1979). The distribution, age effects and disease associations of HLA antigens and other blood group markers in a random sample of an elderly population. Journal of Chronic Diseases, 32, 555.

512) Bender, K., Mayerova, A., Klotzbucher, B., Burckhardt, K., and Hiller, Ch. (1976). No indication of postnatal selection at the HL-A loci. Tissue Antigens, 7, 118.

513) Hansen, H.E., Sparck, J.V., and Larsen, S.O. (1977). An examination of HLA frequencies in three age groups. Tissue Antigens, 10, 49.

514) Macurova, H., Ivanyi, P., Sajdlova, H., and Trojan, J. (1975). HLA antigens in aged persons. Tissue Antigens, 6, 269.

515) Wiener, A.S. (1962). Blood-groups and disease. A critical review. Lancet, i, 813.

516) Manuila, A. (1958). Blood groups and disease - Hard facts and delusions. Journal of the American Medical Association, 167, 2047.

- 517) Mourant, A.E., Kopec', A.C., and Domaniewska - Sobczak, K. (1978). The ABO blood group system. In Blood Groups and Diseases. Oxford: Oxford University Press, p. 146.
- 518) Thomas, J.C., and Hewitt, E.J.C. (1939). Blood groups in health and in mental disease. Journal of Mental Science, 85, 667.
- 519) Masters, A.B. (1967). The distribution of blood groups in psychiatric illness. British Journal of Psychiatry, 113, 1309.
- 520) Mourant, A.E., Kopec', A.C., and Domaniewska - Sobczak, K. (1978). The rhesus blood group system. In Blood Groups and Diseases. Oxford: Oxford University Press, p. 220.
- 521) Woolf, B. (1955). On estimating the relation between blood group and disease. Annals of Human Genetics, 19, 251.
- 522) Emery, A.E.H. (1976). Disease associations. In Methodology in Medical Genetics. Edinburgh: Churchill Livingstone, p. 98.
- 523) Bradley, J. (1977). Transplantation. In Immunology in Medicine, ed., Holborow, E.J., and Reeves, W.G. London: Academic Press, p. 1025.
- 524) Cruz-Coke, R., Paredes, L., and Montenegro, A. (1965).

Blood groups and urinary micro-organisms. *J. Med. Genet.* 2, 185.

525) Robinson, M.G., Tolchin, D., and Halpern, C. (1971). Enteric bacterial agents and the ABO blood groups. *American Journal of Human Genetics*, 23, 135.

526) Foster, M.T., and Labrum, A.H. (1976). Relation of infection with neisseria gonorrhoeae to ABO blood groups. *Journal of Infectious Diseases*, 133, 329.

527) Miler, J.J., Novotny, P., Walker, P.D., Harris, J.R.W., and MacLennan, I.P.B. (1977). Neisseria gonorrhoeae and ABO isohemagglutinins. *Infection and Immunity*, 15, 713.

528) Barua, D., and Paguio, A.S. (1977). ABO blood groups and cholera. *Annals of Human Biology*, 4, 489.

529) Chaudhuri, A., and DasAdhikary, C.R. (1978). Possible role of blood-group secretory substances in the aetiology of cholera. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 72, 664.

530) Mourant, A.E., Kopec, A.C., and Domaniewska - Sobczak, K. (1978). The infective diseases. In *Blood Groups and Diseases*. Oxford: Oxford University Press, p. 18.

531) Muschel, L.H., and Osawa, E. (1959). Human blood

group substance B and *Escherichia coli* 086. *Proc. Soc. Exp. Biol. Med.*, 101, 614.

532) Springer, G.F., Williamson, P., and Brandes, W.C. (1961). Blood group activity of gram-negative bacteria. *J. Exp. Med.*, 113, 1077.

533) Springer, G.F. (1970). Importance of blood-group substances in interactions between man and microbes. *Annals New York Academy of Sciences*, 169, 134.

534) Drach, G.W. (1971). Antigens common to human and bacterial cells: Tolerance or resistance to urinary and surgical infections. *Surgical Forum*, 22, 67.

535) Drach, G.W., Reed, W.P., and Williams, R.C. (1971). Antigens common to human and bacterial cells: Urinary tract pathogens. *Journal of Laboratory and Clinical Medicine*, 78, 725.

536) Henschke, P.J., Bell, D.A., and Cape, R.D.T. (1978). Alzheimer's disease and HLA. *Tissue Antigens*, 12, 132.

537) Cohen, D., Zeller, E., Eisdorfer, C., and Walford, R.L. (1979). Alzheimer's disease and the main histocompatibility complex (HLA system). *Gerontologist*, 19, 57.

538) Walford, R.L., and Hodge, S.E. (1980). HLA distribution

in Alzheimer's disease. In Histocompatibility Testing, ed., Terasaki, P.I. Los Angeles: UCLA Tissue Typing Laboratory, p. 727.

539) Wilcox, C.B., Caspary, E.A., and Behan, P.O. (1980). Histocompatibility antigens in Alzheimer's disease. A preliminary study. European Neurology, 19, 262.

540) Amos, D.B., and Ward, F.E. (1977). Theoretical consideration in the association between HLA and disease. In HLA and Disease, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 269.

541) Zinkernagel, R.M. and Doherty, P.C. (1977). Possible mechanisms of disease-susceptibility association with major transplantation antigens. In HLA and Disease, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 256.

542) Cudworth, A.G., and Festenstein, H. (1978). HLA genetic heterogeneity in diabetes mellitus. British Medical Bulletin, 34, No 3, p. 285.

543) Mayr, W.R. (1975). The SD-3 locus of the HLA system with special reference to T5. In Histocompatibility Testing, ed., Kissmeyer-Nielsen, F. Copenhagen: Munksgaard, p. 330.

544) Hiller, C., Bischoff, M., Schmidt, A., and Bender, K.

(1978). Analysis of the HLA-ABC linkage disequilibrium: Decreasing strength of gametic association with increasing map distance. *Human Genetics*, 41, 301.

545) de Weck, A.L., Blumenthal, M., Yunis, E., and Jeannet, M. (1977). HLA and allergy. In *HLA and Disease*, ed., Dausset, J., and Svejgaard, A. Copenhagen: Munksgaard, p. 196.

546) Buckley, C.E., and Roseman, J.M. (1976). Immunity and survival. *Journal of the American Geriatrics Society*, 24, 241.

547) Williamson, N., Edwards, J.H., Monk, K., McLaughlan, P., Stanworth, D.R., Arnasan, A., Jensson, O., Helgason, T., Kissmeyer-Nielsen, F., Lamm, L.U., van Loghem, E., and de Lange, G. (1979). Anti-tissue antibodies and immunoglobulin levels in relation to HLA and other markers in Icelandic families. *Journal of Immunogenetics*, 6, 223.

548) Vladutiu, A.O., and Rose, N.R. (1971). Autoimmune murine thyroiditis. Relation to histocompatibility (H-2) type. *Science*, 174, 1137.

549) Levine, B.B., Stember, R.H., and Fotino, M. (1972). Ragweed hay fever: Genetic control and linkage to HLA haplotypes. *Science*, 178, 1201.

- 550) Batchelor, J.R., Compston, A., and McDonald, W.I. (1978). The significance of the association between HLA and multiple sclerosis. *British Medical Bulletin*, 34, No. 3, 279.
- 551) Cathala, F., and Brown, P. (1972). The possible viral aetiology of disseminated sclerosis. *Journal of Clinical Pathology*, 25, Suppl. (Roy. Coll. Path.), 6, 141.
- 552) Jersild, C., Ammitzbøll, T., Clausen, J., and Fog, T. (1973). Association between HL-A antigens and measles antibody in multiple sclerosis. *Lancet*, 1, 151.
- 553) Anon. (1979). *Klebsiella* and ankylosing spondylitis- Molecular mimicry? *Lancet*, i, 1012.
- 554) Biddison, W.E., Shearer, G.M. and Shaw, S. (1981). Influenza virus-specific cytotoxic T cells are restricted by multiple HLA-A3-related self antigens: Evidence for recognition of distinct self structures in conjunction with different foreign antigens. *Journal of Immunology*, 127, 6, 2231.
- 555) Pellegrino, M.A., Ferrone, S., Brautbar, C., and Hayflick, L. (1976). Changes in HL-A antigen profiles on SV40 - transformed human fibroblasts. *Experimental Cell Research*, 97, 340.

- 556) Harris, R., Lawler, S.D., and Oliver, R.T.D. (1978). The HLA system in acute leukaemia and Hodgkin's disease. *British Medical Bulletin*, 34, 301.
- 557) Amlot, P., and Lessof, M.H. (1978). Clinical immunology. In *Progress in Clinical Medicine*, ed., Horler, A.R., and Foster, J.B. Edinburgh: Churchill Livingstone, p. 89.
- 558) Harris, R., Zuhrie, S.R., Taylor, G.M., Freeman, C.B., Wentzel, J., Geary, C., and MacIver, J.E. (1977). Influence of HLA, ABO and Rh (D) on survival after remission in acute myelogenous leukaemia. *Lancet*, ii, 653.
- 559) Segal, D.J., Schlaut, J.W., Pabst, H.F., McCoy, E.E., and Dossetor, J.B. (1975). HLA frequencies in Down's syndrome. *Humangenetik*, 27, 45.
- 560) Pereira, R.S., James, D.C.O., and Stern, H. (1978). Correlation between cytomegalovirus infection and HLA-Bw 15. *British Medical Journal*, ii, 126.
- 561) Chalmer, J.E., Mackenzie, J.S., and Stanley, N.F. (1977). Resistance to murine cytomegalovirus linked to the major histocompatibility complex of the mouse. *Journal of General Virology*, 37, 107.
- 562) Gajdusek, D.C. (1972). Slow virus infection and activation of latent infections in aging. In *Advances in*

Gerontological Research, ed., Strehler, B.L. New York and London: Academic Press, Vol. 4, 201.

563) Allison, A.C. (1974). On the role of mononuclear phagocytes in immunity against viruses. Progress in Medical Virology, 18, 15.

564) Oldstone, M.B.A., and Dixon, F.J. (1971). Immune complex disease in chronic viral infections. Journal of Experimental Medicine, 134, 32s.

565) Dixon, F.J. (1977). The role of chronic viral infection in immunologic disease. In Immunology for the Practicing Physician, ed., Schmidtke, J.R., and Ferguson, R.M. New York and London: Plenum Press, p. 101.

566) Allison, A.C. (1972). Immune responses in persistent virus infections. Journal of Clinical Pathology, 25, Suppl. (Roy. Coll. Path.), 6, 121.

567) Hotchin, J., and Collins, D.N. (1964). Glomerulonephritis and late onset disease of mice following neonatal virus infection. Nature, 203, 1357.

568) Hotchin, J.E. (1972). Virus-induced autoimmunity and the aging process. In Tolerance, Autoimmunity and Aging, ed., Sigel, M.M., and Good, R.A. Springfield, Illinois: Charles C. Thomas, p. 132.

- 569) Kaplan, M.E. (1968). Cryoglobulinemia in infectious mononucleosis: Quantitation and characterization of the cryoproteins. *Journal of Laboratory and Clinical Medicine*, 71, 754.
- 570) Wager, O., Rasanen, J.A., Hagman, A., and Klemola, E. (1968). Mixed cryoimmunoglobulinaemia in infectious mononucleosis and cytomegalovirus mononucleosis. *International Archives of Allergy and Applied Immunology*, 34, 345.
- 571) Kantor, G.L., Goldberg, L.S., Johnson, Jr., B.L., Derechin, M.M., and Barnett, E.V. (1970). Immunologic abnormalities induced by postperfusion cytomegalovirus infection. *Annals of Internal Medicine*, 73, 553.
- 572) Hudson, J.B. (1979). The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infections. *Archives of Virology*, 62, 1.
- 573) Stagno, S., Volanakis, J.E., Reynolds, D.W., Stroud, R., and Alford, C.A. (1977). Immune complexes in congenital and natal cytomegalovirus infections of man. *The Journal of Clinical Investigation*, 60, 838.
- 574) Cho, H.J., and Porter, D.D. (1978). Aleutian disease and aleutian disease virus of mink. In *Persistent Viruses*, ed., Stevens, J.G., Todaro, G.J., and Fox, C.F. London:

Academic Press, p. 711.

575) Wisniewski, H.M., and Terry, R.D. (1976). Neuropathology of the aging brain. In *Neurobiology of Aging*, ed., Terry, R.D., and Gershon, S. New York: Raven Press, p. 265.

576) Pien, F.D., Smith, T.F., Taswell, H.F., and Hable, K. A. (1974). Cold-reactive antibodies in a case of congenital cytomegalovirus infection. *American Journal of Clinical Pathology*, 61, 352.

577) Spear, P.G. (1974). Herpesvirus-induced alterations in the composition and function of cell membranes. In *The Neurosciences: Third Study Program*, ed., Schmitt, F.O., and Worden, F.G. Cambridge, Massachusetts and London: The MIT Press, p. 1049.

578) Howard, R.J., and Najarian, J.S. (1974). Cytomegalovirus-induced immune suppression I. Humoral immunity. *Clin. Exp. Immunol.* 18, 109.

579) Howard, R.J., Miller, J., and Najarian, J.S. (1974). Cytomegalovirus-induced immune suppression. II. Cell-mediated immunity. *Clin. Exp. Immunol.*, 18, 119.

580) Schauf, V., Strelkauskas, A.J., and Deveikis, A. (1976). Alteration of lymphocyte subpopulations with cytomegalovirus

infection in infancy. Clin. Exp. Immunol., 26, 478.

581) Sim, M., and Smith, W.T. (1955). Alzheimer's disease confirmed by cerebral biopsy: A therapeutic trial with cortisone and A.C.T.H. Journal of Mental Science, 101, 604.

582) Osborn, J.E., and Medearis, Jr., D.N. (1967). Suppression of interferon and antibody and multiplication of Newcastle disease virus in cytomegalovirus infected mice. Proc. Soc. Exp. Biol. Med., 124, 347.

583) Watson, C.P. (1979). Clinical similarity of Alzheimer and Creutzfeldt-Jakob disease. Annals of Neurology, 6, 368.

584) Ball, M.J. (1980). Features of Creutzfeldt-Jakob disease in brains of patients with familial dementia of Alzheimer type. The Canadian Journal of Neurological Sciences, 7, 51.

585) Pro, J.D., Smith, C.H., and Sumi, S.M. (1980). Presenile Alzheimer disease: Amyloid plaques in the cerebellum. Neurology, 30, 820.

586) Davidson, E.A., and Robertson, E.E. (1955). Alzheimer's disease with acne rosacea in one of identical twins. Journal of Neurology, Neurosurgery and Psychiatry, 18, 72.

587) Hunter, R., Dayan, A.D., and Wilson, J. (1972).

Alzheimer's disease in one monozygotic twin. *Journal of Neurology, Neurosurgery, and Psychiatry*, 35, 707.

588) Sharman, M.G., Watt, D.C., Janota, I., and Carrasco,

L.H. (1979). Alzheimer's disease in a mother and identical twin sons. *Psychological Medicine*, 9, 771.

589) Jacob, H. (1970). Muscular twitchings in Alzheimer's disease. In *Alzheimer's Disease and Related Conditions*, ed., Wolstenholme, G.E.W., and O'Connor, M. London: J. and A. Churchill, p. 75.
